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(54) Title: S. AGALACTIAE ANTIGENS I + II

(57) Abstract: The present invention discloses isolated nucleic acid molecules encoding a hyperimmune serum reactive antigen or a fragment thereof as well as hyperimmune serum reactive antigens or fragments thereof from S. *agalactiae,* methods for isolating such antigens and specific uses thereof.

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S. agalactiae antigens I + II

The present invention relates to isolated nucleic acid molecules, which encode antigens for *Streptococcus agalactiae*, which are suitable for use in preparation of pharmaceutical medicaments for the prevention and treatment of bacterial infections caused by *Streptococcus agalactiae*.

Streptococcus agalactiae is a gram-positive bacterium, and belongs to the Group B Streptococci (GBS) based on its hemolysis pattern in blood agar. The organism is encapsulated, and capsule is an important element of pathogenicity. Capsules are antigenic and form the basis for classifying GBS by serotypes. Nine distinct GBS serotypes have been identified: Ia, Ib, II, III, IV, V, VI, VII and VIII. Most *S. agalactiae* serotypes have been shown to cause serious disease, and the two most common serotypes - type III and V - are estimated to account for the majority of invasive disease worldwide. The ranking and serotype prevalence differs by age group and geographic area. In the US, GBS type III causes more than 50% of infant disease, type V about 40% of nonpregnant adult disease, and type Ia about a third of disease in any patient population.

Streptococcus agalactiae is an important agent of human disease at the extremities of age and in those who have underlying disease. Group B *Streptococci* are the major cause of generalized and focal infections in the newborn infant. GBS is the predominant pathogen in newborns in the US since the 1970's. Bacterial infection can lead to life threatening diseases, such as sepsis, pneumonia and meningitis. Survivers can become permanently handicapped with hearing, learning and visual disabilities. Newborns usually acquire the organism intrapartum or during delivery from their GBS-colonized mothers. In addition, GBS are also a frequent cause of infections in pregnant women and in chronically ill and elderly patients, such as those suffering from diabetes, malignancies, immunodeficiencies, etc., (reviewed by Balter, S. et al. in Gram positive pathogens ed. by Fischetti V.A. et al. ASM Press 2000, pp 154-160).

10-35% of pregnant women are colonized with GBS, but are asymptomatic. However, GBS colonization is important because of the risk of vertical transmission. 50-70% of neonates born to colonized women – that is 5-15% of all newborns - become colonized by GBS during delivery. Colonization is a prerequisite for infection and disease. The most preterm infants are at the highest risk for invasive disease due to low maternal transfer of antibodies and immature immune system. GBS carriage during pregnancy may be chronic, intermittent, or transient. It is difficult to assess the duration of carriage, since women are screened once during a prenatal visit from the late trimester. Several studies suggest that there is a correlation (~90%) between the colonization status in the third trimester and at the time of delivery. Serotyping of the isolates indicates that persistence of the same type is most common (reviewed by Balter, S. et al. in Gram positive pathogens ed. by Fischetti V.A. et al. ASM Press 2000, pp 154-160).

Without preventive intervention, 1 to 2% of all neonates and 15% of neonates born to heavily colonized women develop invasive disease (sepsis, pneumonia and/or meningitis). In the US, GBS infections affect 1-5 newborns/1000 live birth. About 17.000 cases of invasive GBS disease occurred in the US annually, and 7.500 occurred in newborns before prevention. GBS is the most common cause of bacterial meningitis accounting for ~ 40 % of all cases reported in this age group. The overall incidence of invasive GBS disease is 0.2-0.7/100.000 in the US. It is somewhat lower in Europe. Mortality without preventive intervention is 6% with invasive disease, every 16. infected newborn dies and 20% of survivers become permanently handicapped.

The rates of serious group B strep infections are much higher among newborns than among any other age group. Nonetheless, serious group B strep infections occur in other age groups in both men and women. Among non-pregnant adults, rates of serious disease range from 4.1 to 7.2 cases per 100,000 populations. The average death rate for invasive infections (infections where the bacteria have entered a part of the body that is normally not exposed to bacteria) is 8-10% for adult's ages 18-64 and 15-25% for adults 65 years of age and over. Mortality rates are lower among younger adults, and adults who do not have other medical conditions. The rate of serious group B strep disease increases with age. The average age of cases

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in non-pregnant adults is about 60 years old. Most adult group B strep disease occurs in adults who have serious medical conditions. These include: diabetes mellitus; liver disease; history of stroke; history of cancer; or bedsores. Among the elderly, rates of serious group B strep disease are more common among residents of nursing facilities, and among bedridden hospitalized patients. Group B strep disease among non-pregnant adults may often be acquired after recent trauma, or after having certain invasive hospital procedures like surgery ({Farley, M., 2001}; {Jackson, L. et al., 1995}; www.cdc.gov/groupbstrep/).

Direct medical costs of neonatal disease before prevention were $294 million annually and GBS continues to pose a considerable economic burden.

A definitive diagnosis of infection with *Streptococcus agalactiae* generally relies on isolation of the organism from cervical swabs, blood or other normally sterile body sites. Tests are also available to detect capsular polysaccharide antigen in body fluids.

Penicillin G is the treatment of choice for established cases of GBS. Ten days of treatment is recommended for bacteremia, pneumonia and soft tissue infections, while 2-3 weeks is recommended for meningitis and 3-4 weeks for osteomyelitis.

Prevention has been established since 1994 in North America by screening pregnant women for carriage of GBS, taking vaginal and anorectal swabs at 35-37 weeks' gestation, or by identifying risk factors at admission for delivery without cultures. Women who are candidates for prophylaxis are given intrapartum antibiotic therapy during labor to prevent early-onset neonatal disease. This prevention method has decreased the incidence of GBS disease from 1.7 to 0.4/1000 live births between 1993 and 1999 in the US. Although most neonatal GBS disease can be prevented through intrapartum prophylaxis (Penicillin G or Ampicillin), currently available strategies are not ideal, especially for the prevention of late-onset (>7 days of age) infections and disease in premature babies. There are always individuals who escape of screening for carriage due to several reasons, such as intermittent carriers, who are tested negative at wks 32-35, but become positive during delivery, unattendance, negligence, or delivery before screening date (32-35 wks).

In the long run, widespread use of antibiotics usually induces resistant strains that appear after a period of time. Extensive use of Penicillin (every 3-5th women are treated with high dose), and other antibiotics has already been shown to steadily increase the percentage of antibotic resistant clinical isolates (ref). Moreover, efficiency of antibiotic based prevention is not that effective for late onset disease, as it is for early onset (within 48 hrs after delivery). An additional concern is that prevention in susceptible adult populations has not been addressed.

Vaccine development is hindered by the lack of sufficient knowledge about the elements of protective immunity against GBS carriage and disease. The relationship of carriage to the development of natural immunity is poorly understood. In addition, the immunologic mechanism that allows disease to occur in a carrier is ill defined. However, it is suggested that the maternal serum levels of pathogen-specific antibodies are correlated with neonatal GBS disease. It has been firmly established that there is an inverse correlation between maternal anti-capsular polysaccharide antibody levels at delivery and the frequency of invasive neonatal diseases {Campbell, J. et al., 2000}.

Although the group B carbohydrate antigen is common to all strains of GBS, unfortunately, it is not strongly immunogenic and antibodies are not protective from lethal challenge in experimental models. The GBS capsule itself that is made of polysaccharides, is immunogenic and is able to induce protective antibodies. However, this protection is type-specific. Although capsular specific antibodies have been shown to be highly protective, it remains unclear what concentration of these serotype-specific antibodies protect against disease and more recently it has become clear that opsonic activity and avidity of these

antibodies are more critical determinants of protection than concentration.

The importance of surface proteins in human immunity to *S. agalactiae* already has been appreciated. It is apparent that all serotypes express surface proteins with activity relevant to host immune defense. The alpha C protein, beta C protein, Rib and Sip proteins are well-characterized biochemically and genetically, and have also been shown to immunogenic and protective in animal models ({Michel, J. et al., 1991}; {Brodeur, B. et al., 2000}; {Larsson, C. et al., 1999}; {Cheng, Q. et al., 2002}). The major problem with these proteins as vaccine candidates seems to be their variability in prevalenve among the different clinical isolates of GBS. The Rib protein for example is present in serotype III GBS, but missing from type V, which responsible for significant portion of disease worldwide. Some other surface proteins are characterized as being immunogenic, but there is a limited systematic work done to identify most of the immunogenic proteins of GBS.

Thus, there remains a need for an effective treatment to prevent or ameliorate GBS infections. A vaccine could not only prevent infections by GBS, but more specifically prevent or ameliorate colonization of host tissues (esp. in the birth canal), thereby reducing the incidence of transmission from mother to fetus. Reducing the incidence of acute infection and carriage of the organism would lead to prevention of invasive diseases in newborns - pneumonia, bacteremia, meningitis, and sepsis. Vaccines capable of showing cross-protection against the majority of *S. agalactiae* strains causing human infections could also be useful to prevent or ameliorate infections caused by all other streptococcal species, namely groups A, C and G.

A vaccine can contain a whole variety of different antigens. Examples of antigens are whole-killed or attenuated organisms, subfractions of these organisms/tissues, proteins, or, in their most simple form, peptides. Antigens can also be recognized by the immune system in form of glycosylated proteins or peptides and may also be or contain polysaccharides or lipids. Short peptides can be used since for example cytotoxic T-cells (CTL) recognize antigens in form of short usually 8-11 amino acids long peptides in conjunction with major histocompatibility complex (MHC). B-cells can recognize linear epitopes as short as 4-5 amino acids, as well as three-dimensional structures (conformational epitopes). In order to obtain sustained, antigen-specific immune responses, adjuvants need to trigger immune cascades that involve all cells of the immune system. Primarily, adjuvants are acting, but are not restricted in their mode of action, on so-called antigen presenting cells (APCs). These cells usually first encounter the antigen(s) followed by presentation of processed or unmodified antigen to immune effector cells. Intermediate cell types may also be involved. Only effector cells with the appropriate specificity are activated in a productive immune response. The adjuvant may also locally retain antigens and co-injected other factors. In addition the adjuvant may act as a chemoattractant for other immune cells or may act locally and/or systemically as a stimulating agent for the immune system.

Vaccine development since the late 1970s has focused on the capsular polysaccharides, but a safe, effective product is still not available. However, vaccine against *S. agalactiae* is ranked as one of the most important for development and administration to infants and high-risk adults. Currently vaccines against this infection are only in the research stages of development. Efforts are focused on using capsular polysaccharide (CPS) as immunogens, either with or without conjugation to protein {Paoletti, L. et al., 2002}. However, there are several arguments against the use of polysaccharide-based vaccine. Polysaccharides induce IgG2 antibodies, which cross the placenta less efficiently then IgG1 or IgG3 antibodies. It is especially a problem for the most susceptible neonates, the still-borns since placental antibody transfer is low before weeks 32-34. It is estimated that ~10% of deliveries occur before the 34th pregnancy week.

Protein conjugate vaccines are no doubt a great new addition to the amarmatorium in the battle against GBS disease, but the vaccine can contain only a limited number of GBS serotypes and given adequate ecological pressure, replacement disease by non-vaccine serotypes remains a real threat, particularly in

areas with very high disease burden. Morover polysaccharide antigens used for active immunization do not provide immunological memory in humans. Conjugation of CPS to non-GBS related immunogenic protein carriers (e.g. tetanus toxoid, cholera toxin B subunit, etc.) has been shown to beneficial in inducing higher concentrations of antibodies in vaccinees, but it does not provide pathogen-specific B cell and T cell epitopes which would recruit memory B and T cells during a real infection to support the most effective host response. To be able to supplement the CPS vaccines with proteins fulfilling these criteria it is necessary to identify conserved immunogenic GBS-specific surface proteins.

All these insufficiencies suggest that there is a need to develop new generation vaccines composed of proteins, or their derivatives, expressed by all strains under *in vivo* conditions with the ability to induce opsonizing and/or neutralizing antibodies in humans.

There is a great potential for passive antibody-based therapy. There have been already attempts to use human intravenous immunoglobulin (IVIG) preparations for prevention. Recent advances in the technology of monoclonal antibody production provide the means to generate human antibody reagents and reintroduce antibody therapies, while avoiding the toxicities associated with serum therapy. Immunoglobulins are an extremely versatile class of antimicrobial proteins that can be used to prevent and treat emerging infectious diseases. Antibody therapy has been effective against a variety of diverse microorganisms (reviewed in {Burnie, J. et al., 1998}). Anti-GBS mAbs could be given therapeutically to every newborn that develop invasive diseases or preventively to low birth-weight and premature neonates.

During the last decade the immunogenicity and protective capacity of several GBS proteins have been described in animal models and these are now being explored for the development of species-common protein based vaccines. Such proteins are the GBS surface proteins Sip {Brodeur, B. et al., 2000}, rib, ®-protein and {Michel, J. et al., 1991}.

Certain proteins or enzymes displayed on the surface of gram-positive organisms significantly contribute to pathogenesis, are involved in the disease process caused by these pathogens. Often, these proteins are involved in direct interactions with host tissues or in conceiling the bacterial surface from the host defense mechanisms {Navarre, W. et al., 1999}. *S. agalactiae* is not an exception in this regard. Several surface proteins are characterized as virulence factors, important for GBS pathogenicity ((reviewed in (Paoletti L.C. et al. in Gram positive pathogens, ed. by Fischetti V.A et al., ASM Press 2000, pp 137-153); {Paoletti, L. et al., 2002}). If antibodies to these proteins could offer better protection to humans then polysaccharides, they could provide the source of a novel, protein-based GBS vaccine to be used in conjunction with or in place of the more traditional capsular polysaccharise vaccine. The use of some of the above-described proteins as antigens for a potential vaccine as well as a number of additional candidates resulted mainly from a selection based on easiness of identification or chance of availability. There is a demand to identify relevant antigens for *S. agalactiae* in a more comprehensive way.

The present inventors have developed a method for identification, isolation and production of hyperimmune serum reactive antigens from a specific pathogen, especially from *Staphylococcus aureus* and *Staphylococcus epidermidis* (WO 02/059148). However, given the differences in biological property, pathogenic function and genetic background, *Streptococcus agalactiae* is distinctive from *Staphylococcus* strains. Importantly, the selection of sera for the identification of antigens from *S. agalactiae* is different from that applied to the *S. aureus* screens. Four major types of human antibody sources were collected for that purpose. First, healthy pregnant women who were tested negative for cervical and anorectal carriage of GBS. This donor group represents the most important source of antibodies. In addition to their serum samples, human cervical secretions collected with cervical wicks, containing secretory IgA (sIgA) were also used for antigen idntification and validation. The main value of this collection is that sIgA can be considered the major immune effector molecule on mucosal surfaces. Second, healthy pregnant women

colonized with GBS whos newborn remained GBS-free (although with antibiotic prevention). Third, adults below <45 years of age without clinical disease. Four, naïve individuals, youg children between 5 and 10 months of age, after they already lost maternal antibodies and have not acquired GBS-specific ones due to the lack of GBS disease.

To be able to select for relevant serum sources, a series of ELISAs and immunoblotting experiments measuring anti-*S. agalactiae* IgG and IgA antibody levels were performed with bacterial lysates and culture supernatant proteins. Sera from high titer carriers and non-carriers were included in the genomic-based antigen identification. This approach for selection of human sera is basically very different from that used for *S. aureus*, where carriage or non-carriage state couldn't be associated with antibody levels.

The present invention uses high throughput genomic method to identify *in vivo* expressed pathogen-specific proteins with the ability to induce antibodies in humans during the course of infections and colonization.

The genomes of the two bacterial species *S. agalactiae* and *S. aureus* by itself show a number of important differences. The genome of *S. agalactiae* contains app. 2.2 Mb, while *S. aureus* harbours 2.85 Mb. They have an average GC content of 35.7 and 33%, respectively and approximately 30 to 45% of the encoded genes are not shared between the two pathogens. In addition, the two bacterial species require different growth conditions and media for propagation. A list of the most important diseases, which can be inflicted by the two pathogens is presented below. *S. aureus* causes mainly nosocomial, opportunistic infections: impetigo, folliculitis, abscesses, boils, infected lacerations, endocarditis, meningitis, septic arthritis, pneumonia, osteomyelitis, scalded skin syndrome (SSS), toxic shock syndrome. *S. agalactiae* causes mainly neonatal infections and diseases in elderly, such as bacteremia, sepsis, wound infection, osteomyelitis and meningitis.

The complete genome sequence of a capsular serotype III isolate of *S. agalactiae,* designated NEM316 (ATCC 12403) was determined by the random shotgun sequencing strategy (GenBank accession number AL732656; see www.tigr.org/tigrscripts/CMR2/CMRHomePage.spl). {Glaser, P. et al., 2002}.

The problem underlying the present invention was to provide means for the development of medicaments such as vaccines against *S. agalactiae* infection. More particularly, the problem was to provide an efficient, relevant and comprehensive set of nucleic acid molecules or hyperimmune serum reactive antigens from *S. agalactiae* that can be used for the manufacture of said medicaments.

Therefore, the present invention provides an isolated nucleic acid molecule encoding a hyperimmune serum reactive antigen or a fragment thereof comprising a nucleic acid sequence, which is selected from the group consisting of:

a) a nucleic acid molecule having at least 70% sequence identity to a nucleic acid molecule selected from **Seq ID No** 14, 90, 157-216.
b) a nucleic acid molecule which is complementary to the nucleic acid molecule of a),
c) a nucleic acid molecule comprising at least 15 sequential bases of the nucleic acid molecule of a) or b)
d) a nucleic acid molecule which anneals under stringent hybridisation conditions to the nucleic acid molecule of a), b), or c)
e) a nucleic acid molecule which, but for the degeneracy of the genetic code, would hybridise to the nucleic acid molecule defined in a), b), c) or d).

According to a preferred embodiment of the present invention the sequence identity is at least 80%, preferably at least 95%, especially 100%.

Furthermore, the present invention provides an isolated nucleic acid molecule encoding a hyperimmune

serum reactive antigen or a fragment thereof comprising a nucleic acid sequence selected from the group consisting of

a) a nucleic acid molecule having 96 % or more than 96 %, preferably at least 98 %, especially 100 % sequence identity to a nucleic acid molecule selected from **Seq ID No** 1, 3, 5-13, 15, 18-25, 27-31, 33-36, 39-68, 70-85, 92-100, 103-126, 128-145, 147, 149-156, 217, 435-448 and 463-474.
b) a nucleic acid molecule which is complementary to the nucleic acid molecule of a),
c) a nucleic acid molecule comprising at least 15 sequential bases of the nucleic acid molecule of a) or b)
d) a nucleic acid molecule which anneals under stringent hybridisation conditions to the nucleic acid molecule of a), b) or c),
e) a nucleic acid molecule which, but for the degeneracy of the genetic code, would hybridise to the nucleic acid defined in a), b), c) or d).

According to another aspect, the present invention provides an isolated nucleic acid molecule encoding a hyperimmune serum reactive antigen or a fragment thereof comprising a nucleic acid sequence selected from the group consisting of

a) a nucleic acid molecule having 98 % or more than 98%, escpecially 100 % sequence identity to a nucleic acid molecule selected from **Seq ID No** 32, 86, 91, 101, 127.
b) a nucleic acid molecule which is complementary to the nucleic acid of a),
c) a nucleic acid molecule which, but for the degeneracy of the genetic code, would hybridise to the nucleic acid defined in a), b), c) or d).

Preferably, the nucleic acid molecule is DNA or RNA.

According to a preferred embodiment of the present invention, the nucleic acid molecule is isolated from a genomic DNA, especially from a S. *agalactiae* genomic DNA.

According to the present invention a vector comprising a nucleic acid molecule according to any of the present invention is provided.

In a preferred embodiment the vector is adapted for recombinant expression of the hyperimmune serum reactive antigens or fragments thereof encoded by the nucleic acid molecule according to the present invention.

The present invention also provides a host cell comprising the vector according to the present invention.

According to another aspect the present invention further provides a hyperimmune serum-reactive antigen comprising an amino acid sequence being encoded by a nucleic acid molecule according to the present invention.

In a preferred embodiment the amino acid sequence (polypeptide) is selected from the group consisting of **Seq ID No** 231, 307, 374-433.

In another preferred embodiment the amino acid sequence (polypeptide) is selected from the group consisting of **Seq ID No** 218, 220, 222-230, 232, 235-242, 244-248, 250-253, 256-285, 287-302, 309-317, 320-343, 345-362, 364, 366-373, 434, 449-462 and 475-486.

In a further preferred embodiment the amino acid sequence (polypeptide) is selected from the group consisting of **Seq ID No** 249, 303, 308, 318, 344.

According to a further aspect the present invention provides fragments of hyperimmune serum-reactive antigens selected from the group consisting of peptides comprising amino acid sequences of column

"predicted immunogenic aa" and "location of identified immunogenic region" of Table **1A, especially peptides comprising amino acid** 4-20, 35-44, 65-70, 73-87, 92-98, 112-137, 152-161, 177-186, 193-200, 206-213, 229-255, 282-294, 308-313, 320-326, 349-355, 373-384, 388-406, 420-425 and 115-199 of **Seq ID No** 218; 5-24, 35-41, 44-70, 73-89, 103-109, 127-143, 155-161, 185-190, 192-207, 212-219, 246-262, 304-336, 372-382, 384-393, 398-407, 412-418, 438-444, 1-75, 76-161 and 164-239 of **Seq ID No** 219; 4-10, 16-58, 60-71, 77-92, 100-126, 132-146, 149-164, 166-172, 190-209, 214-220, 223-229, 241-256, 297-312, 314-319, 337-343, 351-359, 378-387, 398-418, 421-428, 430-437, 440-448, 462-471, 510-519, 525-536, 552-559, 561-568, 573-582, 596-602, 608-630, 637-649, 651-665, 681-702, 714-732, 739-745, 757-778, 790-805, 807-815, 821-829, 836-842, 846-873, 880-903, 908-914, 916-923, 931-940, 943-948, 956-970, 975-986, 996-1015, 1031-1040, 1051-1069, 1072-1095, 1114-1119, 1130-1148, 1150-1157, 1169-1176, 1229-1238 and 802-812 of **Seq ID No** 220; 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12-28, 41-91, 98-107, 112-120, 125-131, 151-193, 215-221, 240-250, 263-280 and 128-138 of **Seq ID No** 368; 16-24, 32-38, 46-62, 68-81, 90-105, 127-133, 144-150, 160-166, 178-184, 186-202, 210-219, 232-240, 252-258, 264-273, 293-324, 337-344, 349-357, 360-369, 385-398, 410-416, 419-427, 441-449, 458-476, 508-515, 523-539, 544-549, 562-569, 571-579, 96-109 and 127-139 of **Seq ID No** 369; 19-25, 28-34, 56-61, 85-97, 110-116 and 39-53 of **Seq ID No** 370; 4-37, 41-50, 62-72, 91-97, 99-109, 114-125, 136-141, 149-158, 160-166, 201-215 and 27-225 of **Seq ID No** 371; 15-31, 44-51, 96-105, 122-130, 149-157, 162-168, 178-183, 185-192, 198-204, 206-213, 221-234, 239-245, 248-255, 257-266, 289-335, 349-357, 415-422, 425-441, 448-454, 462-468 and 463-481 of **Seq ID No** 372; 5-31, 39-55, 63-72, 76-99, 106-155, 160-177, 179-199, 207-217, 223-240, 245-255, 261-267, 294-316, 321-343, 354-378, 382-452, 477-488, 529-536, 555-569, 584-591, 593-612, 620-627, 632-640, 647-654, 671-680, 698-704, 723-730, 732-750, 769-775, 781-788, 822-852 and 505-525 of **Seq ID No** 373; 3-18 of **Seq ID No** 374; 4-14 and 12-24 of **Seq ID No** 375; 4-11, 22-30 and 12-25 of **Seq ID No** 376; 5-12 and 4-18 of **Seq ID No** 377; 4-28 and 7-14 of **Seq ID No** 378; 6-16 and 8-16 of **Seq ID No** 379; 4-15, 18-33 and 24-36 of **Seq ID No** 380; 4-10, 16-21 and 20-31 of **Seq ID No** 381; 6-19 of **Seq ID No** 382; 11-18 and 3-10 of **Seq ID No** 383; 13-24 and 3-15 of **Seq ID No** 384; 15-27 and 7-16 of **Seq ID No** 385; 11-16 and 1-15 of **Seq ID No** 386; 4-16 and 9-21 of **Seq ID No** 387; 4-24, 40-48, 54-67 and 22-39 of **Seq ID No** 388; 6-30, 34-55, 62-68, 78-106 and 68-74 of **Seq ID No** 389; 3-14 of **Seq ID No** 390; 9-19 and 6-21 of **Seq ID No** 391; 4-17 and 1-9 of **Seq ID No** 392; 5-30 and 1-8 of **Seq ID No**

393; 4-16, 23-46, 51-56 and 45-55 of **Seq ID No** 394; 7-16 of **Seq ID No** 395; 2-14 of **Seq ID No** 396; 4-36, 43-65 and 50-62 of **Seq ID No** 397; 10-30 and 14-21 of **Seq ID No** 398; 9-17 and 1-10 of **Seq ID No** 399; 4-12 and 3-16 of **Seq ID No** 400; 4-15 and 5-23 of **Seq ID No** 401; 10-21 of **Seq ID No** 402; 6-16 of **Seq ID No** 403; 4-29, 31-38 and 2-14 of **Seq ID No** 404; 4-35 and 33-42 of **Seq ID No** 405; 2-17 of **Seq ID No** 406; 9-18, 30-35 and 15-33 of **Seq ID No** 407; 4-9 and 6-12 of **Seq ID No** 408; 3-17 of **Seq ID No** 409; 12-21, 37-44, 52-61, 72-80 and 38-48 of **Seq ID No** 410; 4-10, 29-44, 54-61, 69-78 and 13-27 of **Seq ID No** 411; 13-23, 36-53 and 2-15 of **Seq ID No** 412; 4-25, 28-46, 56-72, 81-99, 120-132, 134-142, 154-160 and 129-141 of **Seq ID No** 413; 4-15, 24-33, 35-41, 64-86 and 21-33 of **Seq ID No** 414; 9-15 and 4-13 of **Seq ID No** 415; 4-11, 13-19, 34-48 and 15-32 of **Seq ID No** 416; 4-21 and 11-31 of **Seq ID No** 417; 23-57 and 38-50 of **Seq ID No** 418; 4-32 and 3-13 of **Seq ID No** 419; 4-10, 13-25, 32-42, 56-68, 72-84 and 26-38 of **Seq ID No** 420; 4-20, 31-48, 52-58, 65-71, 80-93, 99-108, 114-123 and 37-49 of **Seq ID No** 421; 6-12, 14-20 and 3-25 of **Seq ID No** 422; 14-25, 27-38 and 5-14 of **Seq ID No** 423; 4-41, 57-105, 109-118, 123-136, 144-152 and 86-99 of **Seq ID No** 424; 6-19 of **Seq ID No** 425; 2-19 of **Seq ID No** 426; 14-47 and 1-14 of **Seq ID No** 427; 4-21, 29-44 and 2-18 of **Seq ID No** 428; 23-29 and 10-28 of **Seq ID No** 429; 6-16, 22-36 and 11-22 of **Seq ID No** 430; 4-19, 30-44 and 18-27 of **Seq ID No** 431; 5-15, 37-45, 58-65 and 38-47 of **Seq ID No** 432; 4-15, 23-34 and 4-15 of **Seq ID No** 433; 30-36, 44-54, 79-85, 101-114, 138-152, 154-164, 170-175, 179-200, 213-220, 223-240, 243-255, 258-264, 268-284 and 10-28 of **Seq ID No** 434; **the peptides comprising amino acid sequences of column "Identical region" of the Table 1B, especially peptides comprising amino acid** 210-226 and 738-753 of **Seq ID No** 449; 326-344, 326-348, 338-354, 371-392, 801-809 and 877-901 of **Seq ID No** 450; 893-906 of **Seq ID No** 451; 51-69 of **Seq ID No** 452; 110-125 of **Seq ID No** 453; 291-305 of **Seq ID No** 454; 210-226 and 738-753 of **Seq ID No** 455; 326-344, 326-348, 338-354, 371-392, 801-809 and 877-901 of **Seq ID No** 456; 893-906 of **Seq ID No** 457; 51-69 of **Seq ID No** 458; 110-125 of **Seq ID No** 459; 291-305 of **Seq ID No** 460; 32-44 of **Seq ID No** 461; 399-410 of **Seq ID No** 462; the serum reactive epitopes **as specified in the column of "aa from" to "aa to"** of Table **2, especially peptides comprising amino acid** 120-143, 138-161 and 156-179 of **Seq ID No** 218; 110-129 and 168-184 of **Seq ID No** 219; 74-90 of **Seq ID No** 222; 759-773 of **Seq ID No** 223; 237-260 of **Seq ID No** 224; 265-284 of **Seq ID No** 225; 65-74 of **Seq ID No** 226; 41-50 of **Seq ID No** 227; 163-174 of **Seq ID No** 229; 26-37 of **Seq ID No** 230; 174-189 of **Seq ID No** 232; 240-256 of **Seq ID No** 234; 285-297 of **Seq ID No** 236; 238-247 of **Seq ID No** 238; 491-519 of **Seq ID No** 239; 114-140 of **Seq ID No** 243; 267-284 of **Seq ID No** 250; 439-453 of **Seq ID No** 252; 162-178 of **Seq ID No** 253; 347-364 of **Seq ID No** 254; 699-715 of **Seq ID No** 255; 60-71 of **Seq ID No** 256; 244-257 of **Seq ID No** 257; 44-63 and 57-76 of **Seq ID No** 258; 185-196 of **Seq ID No** 260; 119-129 of **Seq ID No** 263; 182-195 of **Seq ID No** 266; 32-44 and 424-442 of **Seq ID No** 267; 247-256 of **Seq ID No** 268; 678-694, 785-805, 55-77 and 72-94 of **Seq ID No** 269; 210-226 of **Seq ID No** 281; 37-59 of **Seq ID No** 289; 13-29 of **Seq ID No** 296; 136-159 of **Seq ID No** 348; 205-222 of **Seq ID No** 349; 99-110 of **Seq ID No** 350; 160-176 of **Seq ID No** 351; 457-470 of **Seq ID No** 355; 221-237 of **Seq ID No** 356; 167-190 of **Seq ID No** 357; 96-120 of **Seq ID No** 361; 399-417, 503-519 and 544-563 of **Seq ID No** 364; 46-68, 159-183 and 184-198 of **Seq ID No** 371; 463-481 of **Seq ID No** 372; **the immunogenic epitopes as specified in the column of "aa from" to "aa to" of Table 4;** especially peptides comprising amino acid 110-129 and 168-184 of **Seq ID No** 219; 877-901, 333-354, 326-344 and 801-809 of **Seq ID No** 277; 1-54 of **Seq ID No** 347; 544-563, 31-51, 107-119, 399-417 and 503-519 of **Seq ID No** 364; 120-198 of **Seq ID No** 218; 20-35 of **Seq ID No** 219; 118-201 of **Seq ID No** 221; 48-132 of **Seq ID No** 242; 118-136 of **Seq ID No** 249; 162-178 of **Seq ID No** 253; 347-364 of **Seq ID No** 254; 699-715 of **Seq ID No** 255; 50-76 of **Seq ID No** 258; 785-819 and 44-128 of **Seq ID No** 269; 90-128 of **Seq ID No** 274; 314-384 of **Seq ID No** 289; 327-349 of **Seq ID No** 293; 242-314, 405-478 and 23-100 of **Seq ID No** 304; 129-210 of **Seq ID No** 305; 162-188 of **Seq ID No** 307; 750-772 of **Seq ID No** 310; 1-56 of **Seq ID No** 335; 322-337 of **Seq ID No** 337; 72-90 of **Seq ID No** 339; 374-395 of **Seq ID No** 345; 136-159 of **Seq ID No** 348; 141-164 of **Seq ID No** 358; 96-157 of **Seq ID No** 361; 1-82 of **Seq ID No** 363; 489-556 of **Seq ID No** 364; 159-183 and 49-133 of **Seq ID No** 371; **The peptides comprising amino acid sequences of column "predicted immunogenic aa" and "location of identified immunogenic region (aa)" of Table 5, especially peptides comprising amino acid** 4-26, 35-41, 53-61, 73-84, 103-108, 114-120, 140-146, 156-162, 192-208, 214-219, 227-233, 239-252, 260-268, 284-297, 1-48 and 113-133 of **Seq ID No** 475; 4-27, 38-44, 50-56, 59-64, 72-79, 83-89, 92-97, 108-116, 123-148, 152-167, 183-196, 200-220, 232-244, 255-261, 265-274, 282-302, 309-317, 1-79 and 231-302 of **Seq ID No** 476; 6-28, 66-72, 85-105, 115-121, 144-151, 160-170, 176-185, 223-230, 252-288, 296-

310, 319-333, 367-374, 458-464, 471-480, 483-488, 520-528, 530-549, 559-564, 593-601, 606-616, 636-643, 655-662, 676-682, 684-699, 719-726, 735-750, 757-764, 777-785, 799-810, 812-843, 846-853, 868-873, 880-889, 891-899, 909-929, 934-940, 963-969, 998-1004, 1007-1014, 1016-1022, 1030-1046, 1-80 and 808-821 of **Seq ID No** 477; 7-24, 35-41, 75-81, 91-114, 122-132, 137-144, 148-156, 183-192, 194-200, 212-228, 233-238, 251-258, 275-295, 326-332, 337-346, 1-79 and 305-321 of **Seq ID No** 478; 31-38, 42-52, 66-72, 86-92, 98-104, 115-122, 127-146, 154-164, 169-187, 198-212, 225-237, 255-269, 13-92 and 135-142 of **Seq ID No** 479; 4-36, 39-49, 63-69, 71-77, 81-88, 123-131, 133-139, 160-169, 174-180, 188-194, 210-217, 273-278, 289-300, 317-334, 336-341, 383-401, 425-438, 1-68, 212-270 and 402-446 of **Seq ID No** 480; 21-29, 31-42, 49-63, 72-79, 81-93, 112-132, 159-165, 188-195, 197-232, 262-267, 279-286, 294-301, 318-326, 348-366, 381-405, 409-426, 436-465, 471-480, 484-492, 497-505, 521-544, 554-561, 567-577, 581-589, 601-609, 611-622, 636-651, 653-667, 669-685, 700-708, 716-722, 729-744, 749-766, 780-786, 789-811, 814-864, 1-57 and 84-106 of **Seq ID No** 481; 6-24, 35-48, 57-63, 72-78, 87-92, 113-119, 123-137, 147-153, 173-181, 212-233 and 1-124 of **Seq ID No** 482; 13-34, 62-69, 78-83, 86-91, 98-104, 107-115, 146-159, 179-188, 195-205, 209-221, 226-233, 239-253, 276-282, 284-294, 297-308, 331-354, 375-382, 388-399, 421-433, 449-458, 464-469, 472-491, 508-513, 525-531, 534-550, 575-593, 601-618, 629-635, 654-661, 666-680, 706-721, 723-740, 771-805, 810-830, 845-851 and 1-84 of **Seq ID No** 483; 4-32, 45-64, 73-83, 86-92, 100-111, 125-147, 157-163, 170-175, 177-188, 226-232, 245-252, 258-274, 320-335, 348-359 and 1-71 of **Seq ID No** 484; 13-40, 43-71, 76-83, 87-101, 109-119, 125-156, 162-175, 182-219, 226-232, 240-262, 270-287, 306-318, 326-342, 344-408, 414-444, 449-456 and 1-51 of **Seq ID No** 485; 4-16, 18-34, 45-54, 99-108, 134-140, 203-212, 241-257, 266-274, 279-291, 308-315, 330-336, 355-370, 374-382, 402-410, 428-455, 466-472, 474-480, 531-554, 560-566, 572-580, 597-618, 632-660, 664-674, 676-685, 691-705, 708-735, 750-768, 1-87 and 342-480 of **Seq ID No** 486; **The serum reactive epitopes as specified in the column of "aa from" to "aa to" of Table 6, especially peptides comprising amino acid** 115-132 and 1-26 of **Seq ID No** 475; 33-55 of **Seq ID No** 476; 1-25 of **Seq ID No** 478; 37-61 of **Seq ID No** 479; 1-24 of **Seq ID No** 480; 1-23 of **Seq ID No** 481; 46-60 of **Seq ID No** 482; 1-28, 23-50 and 45-71 of **Seq ID No** 483; 1-22 and 17-38 of **Seq ID No** 484; 1-22 and 17-38 of **Seq ID No** 485; 1-27, 22-47 and 422-447 of **Seq ID No** 486; **The immunogenic epitopes as specified in the column of "aa from" to "aa to" of Table 7, especially peptides comprising amino acid** 115-132 and 1-47 of **Seq ID No** 475; 1-55 of **Seq ID No** 476; 22-85 of **Seq ID No** 477; 307-320 and 1-44 of **Seq ID No** 478; 15-76 and 40-92 of **Seq ID No** 479; 1-59, 213-269 and 403-445 of **Seq ID No** 480; 1-56 and 85-105 of **Seq ID No** 481; 37-121 of **Seq ID No** 482; 1-71 of **Seq ID No** 483; 1-38 of **Seq ID No** 484; 1-38 of **Seq ID No** 485; 1-47 of **Seq ID No** 486.

The present invention also provides a process for producing a *S. agalactiae* hyperimmune serum reactive antigen or a fragment thereof according to the present invention comprising expressing one or more of the nucleic acid molecules according to the present invention in a suitable expression system.

Moreover, the present invention provides a process for producing a cell, which expresses a *S agalactiae* hyperimmune serum reactive antigen or a fragment thereof according to the present invention comprising transforming or transfecting a suitable host cell with the vector according to the present invention.

According to the present invention a pharmaceutical composition, especially a vaccine, comprising a hyperimmune serum-reactive antigen or a fragment thereof as defined in the present invention or a nucleic acid molecule as defined in the present invention is provided.

In a preferred embodiment the pharmaceutical composition further comprises an immunostimulatory substance, preferably selected from the group comprising polycationic polymers, especially polycationic peptides, immunostimulatory deoxynucleotides (ODNs), peptides containing at least two LysLeuLys motifs, especially KLKLSKLK, neuroactive compounds, especially human growth hormone, alumn, Freund's complete or incomplete adjuvants or combinations thereof.

In a more preferred embodiment the immunostimulatory substance is a combination of either a polycationic polymer and immunostimulatory deoxynucleotides or of a peptide containing at least two

LysLeuLys motifs and immunostimulatory deoxynucleotides.

In a still more preferred embodiment the polycationic polymer is a polycationic peptide, especially polyarginine.

According to the present invention the use of a nucleic acid molecule according to the present invention or a hyperimmune serum-reactive antigen or fragment thereof according to the present invention for the manufacture of a pharmaceutical preparation, especially for the manufacture of a vaccine against *S. agalactiae* infection, is provided.

Also an antibody, or at least an effective part thereof, which binds at least to a selective part of the hyperimmune serum-reactive antigen or a fragment thereof according to the present invention, is provided herewith.

In a preferred embodiment the antibody is a monoclonal antibody.

In another preferred embodiment the effective part of the antibody comprises Fab fragments.

In a further preferred embodiment the antibody is a chimeric antibody.

In a still preferred embodiment the antibody is a humanized antibody.

The present invention also provides a hybridoma cell line, which produces an antibody according to the present invention.

Moreover, the present invention provids a method for producing an antibody according to the present invention, characterized by the following steps:

- initiating an immune response in a non-human animal by administrating an hyperimmune serum-reactive antigen or a fragment thereof, as defined in the invention, to said animal,
- removing an antibody containing body fluid from said animal, and
- producing the antibody by subjecting said antibody containing body fluid to further purification steps.

Accordingly, the present invention also provides a method for producing an antibody according to the present invention, characterized by the following steps:

- initiating an immune response in a non-human animal by administrating an hyperimmune serum-reactive antigen or a fragment thereof, as defined in the present invention, to said animal,
- removing the spleen or spleen cells from said animal,
- producing hybridoma cells of said spleen or spleen cells,
- selecting and cloning hybridoma cells specific for said hyperimmune serum-reactive antigens or a fragment thereof,
- producing the antibody by cultivation of said cloned hybridoma cells and optionally further purification steps.

The antibodies provided or produced according to the above methods may be used for the preparation of a medicament for treating or preventing *S. agalactiae* infections.

According to another aspect the present invention provides an antagonist, which binds to a hyperimmune serum-reactive antigen or a fragment thereof according to the present invention.

Such an antagonist capable of binding to a hyperimmune serum-reactive antigen or fragment thereof according to the present invention may be identified by a method comprising the following steps:

a) contacting an isolated or immobilized hyperimmune serum-reactive antigen or a fragment thereof according to the present invention with a candidate antagonist under conditions to permit binding of said candidate antagonist to said hyperimmune serum-reactive antigen or fragment, in the presence of a component capable of providing a detectable signal in response to the binding of the candidate antagonist to said hyperimmune serum reactive antigen or fragment thereof; and
b) detecting the presence or absence of a signal generated in response to the binding of the antagonist to the hyperimmune serum reactive antigen or the fragment thereof.

An antagonist capable of reducing or inhibiting the interaction activity of a hyperimmune serum-reactive antigen or a fragment thereof according to the present invention to its interaction partner may be identified by a method comprising the following steps:

a) providing a hyperimmune serum reactive antigen or a hyperimmune fragment thereof according to the present invention,
b) providing an interaction partner to said hyperimmune serum reactive antigen or a fragment thereof, especially an antibody according to the present invention,
c) allowing interaction of said hyperimmune serum reactive antigen or fragment thereof to said interaction partner to form an interaction complex,
d) providing a candidate antagonist,
e) allowing a competition reaction to occur between the candidate antagonist and the interaction complex ,
f) determining whether the candidate antagonist inhibits or reduces the interaction activities of the hyperimmune serum reactive antigen or the fragment thereof with the interaction partner.

The hyperimmune serum reactive antigens or fragments thereof according to the present invention may be used for the isolation and/or purification and/or identification of an interaction partner of said hyperimmune serum reactive antigen or fragment thereof.

The present invention also provides a process for *in vitro* diagnosing a disease related to expression of a hyperimmune serum-reactive antigen or a fragment thereof according to the present invention comprising determining the presence of a nucleic acid sequence encoding said hyperimmune serum reactive antigen or fragment thereof according to the present invention or the presence of the hyperimmune serum reactive antigen or fragment thereof according to the present invention.

The present invention also provides a process for *in vitro* diagnosis of a bacterial infection, especially a *S. agalactiae* infection, comprising analyzing for the presence of a nucleic acid sequence encoding said hyperimmune serum reactive antigen or fragment thereof according to the present invention or the presence of the hyperimmune serum reactive antigen or fragment thereof according to the present invention.

Moreover, the present invention provides the use of a hyperimmune serum reactive antigen or fragment thereof according to the present invention for the generation of a peptide binding to said hyperimmune serum reactive antigen or fragment thereof, wherein the peptide is an anticaline.

The present invention also provides the use of a hyperimmune serum-reactive antigen or fragment thereof according to the present invention for the manufacture of a functional nucleic acid, wherein the functional nucleic acid is selected from the group comprising aptamers and spiegelmers.

The nucleic acid molecule according to the present invention may also be used for the manufacture of a functional ribonucleic acid, wherein the functional ribonucleic acid is selected from the group comprising ribozymes, antisense nucleic acids and siRNA.

The present invention advantageously provides an efficient, relevant and comprehensive set of isolated nucleic acid molecules and their encoded hyperimmune serum reactive antigens or fragments thereof identified from *S. agalactiae* using an antibody preparation from multiple human plasma pools and surface expression libraries derived from the genome of *S. agalactiae*. Thus, the present invention fulfils a widely felt demand for *S. agalactiae* antigens, vaccines, diagnostics and products useful in procedures for preparing antibodies and for identifying compounds effective against *S. agalactiae* infection.

An effective vaccine should be composed of proteins or polypeptides, which are expressed by all strains and are able to induce high affinity, abundant antibodies against cell surface components of S. *agalactiae*. The antibodies should be IgG1 and/or IgG3 for opsonization, and any IgG subtype and IgA for neutralisation of adherence and toxin action. A chemically defined vaccine must be definitely superior compared to a whole cell vaccine (attenuated or killed), since components of S. *agalactiae*, which cross-react with human tissues or inhibit opsonization can be eliminated, and the individual proteins inducing protective antibodies and/or a protective immune response can be selected.

The approach, which has been employed for the present invention, is based on the interaction of GBS proteins or peptides with the antibodies present in human sera. The antibodies produced against *S. agalactiae* by the human immune system and present in human sera are indicative of the *in vivo* expression of the antigenic proteins and their immunogenicity. In addition, the antigenic proteins as identified by the bacterial surface display expression libraries using pools of pre-selected sera, are processed in a second and third round of screening by individual selected or generated sera. Thus the present invention supplies an efficient, relevant, comprehensive set of GBS antigens as a pharmaceutical composition, especially a vaccine preventing infection by *S. agalactiae*.

In the antigen identification program for identifying a comprehensive set of antigens according to the present invention, at least two different bacterial surface expression libraries are screened with several serum pools or plasma fractions or other pooled antibody containing body fluids (antibody pools). The antibody pools are derived from a serum collection, which has been tested against antigenic compounds of *S. agalactiae*, such as whole cell extracts and culture supernatant proteins. Preferably, three distinct serum collections are used, obtained ad 1. from healthy pregnant women tested negative for cervical and anorectal carriage of GBS, ad 2. healthy pregnant women tested positive for cervical and/or anorectal carriage of GBS who's newborn remained GBS-free (although with antibiotic prevention), ad 3. from adults below <45 years of age without clinical disease. Sera have to react with multiple GBS-specific antigens in order to be considered hyperimmune and therefore relevant in the screening method applied for the present invention.

The expression libraries as used in the present invention should allow expression of all potential antigens, e.g. derived from all secreted and surface proteins of *S. agalactiae*. Bacterial surface display libraries will be represented by a recombinant library of a bacterial host displaying a (total) set of expressed peptide sequences of *S. agalactiae* on two selected outer membrane proteins (LamB and FhuA) at the bacterial host membrane {Georgiou, G., 1997}; {Etz, H. et al., 2001}. One of the advantages of using recombinant expression libraries is that the identified hyperimmune serum-reactive antigens may be instantly produced by expression of the coding sequences of the screened and selected clones expressing the hyperimmune serum-reactive antigens without further recombinant DNA technology or cloning steps necessary.

The comprehensive set of antigens identified by the described program according to the present invention is analysed further by one or more additional rounds of screening. Therefore individual antibody preparations or antibodies generated against selected peptides, which were identified as immunogenic are used. According to a preferred embodiment the individual antibody preparations for the second round of screening are derived from pregnant women and non-pregant adults who show an antibody titer above a certain minimum level, for example an antibody titer being higher than 80

percentile, preferably higher than 90 percentile, especially higher than 95 percentile of the human (patient or healthy individual) sera tested. Using such high titer individual antibody preparations in the second screening round allows a very selective identification of the hyperimmune serum-reactive antigens and fragments thereof from *S. agalactiae*.

Following the comprehensive screening procedure, the selected antigenic proteins, expressed as recombinant proteins or *in vitro* translated products, in case it can not be expressed in prokaryotic expression systems, or the identified antigenic peptides (produced synthetically) are tested in a second screening by a series of ELISA and Western blotting assays for the assessment of their immunogenicity with a large human serum collection (minimum ~150 healthy and patients sera).

It is important that the individual antibody preparations (which may also be the selected serum) allow a selective identification of the most promising candidates of all the hyperimmune serum-reactive antigens from all the promising candidates from the first round. Therefore, preferably at least 10 individual antibody preparations (i.e. antibody preparations (e.g. sera) from at least 10 different individuals having suffered from an infection to the chosen pathogen) should be used in identifying these antigens in the second screening round. Of course, it is possible to use also less than 10 individual preparations, however, selectivity of the step may not be optimal with a low number of individual antibody preparations. On the other hand, if a given hyperimmune serum-reactive antigen (or an antigenic fragment thereof) is recognized by at least 10 individual antibody preparations, preferably at least 30, especially at least 50 individual antibody preparations, identification of the hyperimmune serum-reactive antigen is also selective enough for a proper identification. Hyperimmune serum-reactivity may of course be tested with as many individual preparations as possible (e.g. with more than 100 or even with more than 1,000).

Therefore, the relevant portion of the hyperimmune serum-reactive antibody preparations according to the method of the present invention should preferably be at least 10, more preferred at least 30, especially at least 50 individual antibody preparations. Alternatively (or in combination) hyperimmune serum-reactive antigens may preferably be also identified with at least 20%, preferably at least 30%, especially at least 40% of all individual antibody preparations used in the second screening round.

According to a preferred embodiment of the present invention, the sera from which the individual antibody preparations for the second round of screening are prepared (or which are used as antibody preparations), are selected by their titer against *S. agalactiae* (e.g. against a preparation of this pathogen, such as a lysate, cell wall components and recombinant proteins). Preferably, some are selected with a total IgA titer above 300 U, especially above 500 U, and/or an IgG titer above 5,000 U, especially above 10,000 U (U = units, calculated from the OD_{405nm} reading at a given dilution) when the whole organism (total lysate or whole cells) is used as antigen in the ELISA.

The antibodies produced against streptococci by the human immune system and present in human sera are indicative of the *in vivo* expression of the antigenic proteins and their immunogenicity. The recognition of linear epitopes recognized by serum antibodies can be based on sequences as short as 4-5 amino acids. Of course it does not necessarily mean that these short peptides are capable of inducing the given antibody *in vivo*. For that reason the defined epitopes, polypeptides and proteins are further to be tested in animals (mainly in mice) for their capacity to induce antibodies against the selected proteins *in vivo*.

The preferred antigens are located on the cell surface or secreted, and are therefore accessible extracellularly. Antibodies against cell wall proteins are expected to serve multiple purposes: to inhibit adhesion, to interfere with nutrient acquisition, to inhibit immune evasion nand to promote phagocytosis {Hornef, M. et al., 2002}. Antibodies against secreted proteins are beneficial in neutralisation of their function as toxin or virulence component. It is also known that bacteria communicate with each other

through secreted proteins. Neutralizing antibodies against these proteins will interrupt growth-promoting cross-talk between or within streptococcal species. Bioinformatic analyses (signal sequences, cell wall localisation signals, transmembrane domains) proved to be very useful in assessing cell surface localisation or secretion. The experimental approach includes the isolation of antibodies with the corresponding epitopes and proteins from human serum, and the generation of immune sera in mice against (poly) peptides selected by the bacterial surface display screens. These sera are then used in a third round of screening as reagents in the following assays: cell surface staining of *S. agalactiae* grown under different conditions (FACS or microscopy), determination of neutralizing capacity (toxin, adherence), and promotion of opsonization and phagocytosis (*in vitro* phagocytosis assay).

For that purpose, bacterial *E. coli* clones are directly injected into mice and immune sera are taken and tested in the relevant *in vitro* assay for functional opsonic or neutralizing antibodies. Alternatively, specific antibodies may be purified from human or mouse sera using peptides or proteins as substrate.

Host defence against *S. agalactiae* relies mainly on opsonophagocytic killing mechanism. Inducing high affinity antibodies of the opsonic and neutralizing type by vaccination helps the innate immune system to eliminate bacteria and toxins. This makes the method according to the present invention an optimal tool for the identification of GBS antigenic proteins.

The skin and mucous membranes are formidable barriers against invasion by streptococci. However, once the skin or the mucous membranes are breached the first line of non-adaptive cellular defence begins its co-ordinate action through complement and phagocytes, especially the polymorphonuclear leukocytes (PMNs). These cells can be regarded as the cornerstones in eliminating invading bacteria. As *Streptococcus agalactiae* is a primarily extracellular pathogen, the major anti-streptococcal adaptive response comes from the humoral arm of the immune system, and is mediated through three major mechanisms: promotion of opsonization, toxin neutralisation, and inhibition of adherence. It is believed that opsonization is especially important, because of its requirement for an effective phagocytosis. For efficient opsonization the microbial surface has to be coated with antibodies and complement factors for recognition by PMNs through receptors to the Fc fragment of the IgG molecule or to activated C3b. After opsonization, streptococci are phagocytosed and killed. Antibodies bound to specific antigens on the cell surface of bacteria serve as ligands for the attachment to PMNs and to promote phagocytosis. The very same antibodies bound to the adhesins and other cell surface proteins are expected to neutralize adhesion and prevent colonization. The selection of antigens as provided by the present invention is thus well suited to identify those that will lead to protection against infection in an animal model or in humans.

According to the antigen identification method used herein, the present invention can surprisingly provide a set of comprehensive novel nucleic acids and novel hyperimmune serum reactive antigens and fragments thereof of *S. agalactiae*, among other things, as described below. According to one aspect, the invention particularly relates to the nucleotide sequences encoding hyperimmune serum reactive antigens which sequences are set forth in the Sequence listing **Seq ID No**: 1-217, 435-448 and 463-474 and the corresponding encoded amino acid sequences representing hyperimmune serum reactive antigens are set forth in the Sequence Listing **Seq ID No** 218-434, 449-462 and 475-486.

In a preferred embodiment of the present invention, a nucleic acid molecule is provided which exhibits 70% identity over their entire length to a nucleotide sequence set forth with **Seq ID No** 14, 90, 157-216.. Most highly preferred are nucleic acids that comprise a region that is at least 80% or at least 85% identical over their entire length to a nucleic acid molecule set forth with **Seq ID No** 14, 90, 157-216. In this regard, nucleic acid molecules at least 90%, 91%, 92%, 93%, 94%, 95%, or 96% identical over their entire length to the same are particularly preferred. Furthermore, those with at least 97% are highly preferred, those with at least 98% and at least 99% are particularly highly preferred, with at least 99% or 99.5% being the more preferred, with 100% identity being especially preferred. Moreover, preferred embodiments in this respect are nucleic acids which encode hyperimmune serum reactive antigens or fragments thereof

(polypeptides) which retain substantially the same biological function or activity as the mature polypeptide encoded by said nucleic acids set forth in the **Seq ID No** 14, 90, 157-216.

Identity, as known in the art and used herein, is the relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, identity also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. Identity can be readily calculated. While there exist a number of methods to measure identity between two polynucleotide or two polypeptide sequences, the term is well known to skilled artisans (e.g. *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987). Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity are codified in computer programs. Preferred computer program methods to determine identity between two sequences include, but are not limited to, GCG program package {Devereux, J. et al., 1984}, BLASTP, BLASTN, and FASTA {Altschul, S. et al., 1990}.

According to another aspect of the invention, nucleic acid molecules are provided which exhibit 96 % or more than 96 %, especially 100 % sequence identity to the nucleic acid sequence set forth with **Seq ID No** 1, 3, 5-13, 15, 18-25, 27-31, 33-36, 39-68, 70-85, 92-100, 103-126, 128-145, 147, 149-156, 217, 435-448 and 463-474.

According to a further aspect of the present invention, nucleic acid molecules having 98 % or more than 98%, escpecially 100 % sequence identity to a nucleic acid molecule are provided which are identical to the nucleic acid sequences set forth with **Seq ID No** 32, 86, 91, 101, 127.

The nucleic acid molecules according to the present invention can as a second alternative also be a nucleic acid molecule which is at least essentially complementary to the nucleic acid described as the first alternative above. As used herein complementary means that a nucleic acid strand is base pairing via Watson-Crick base pairing with a second nucleic acid strand. Essentially complementary as used herein means that the base pairing is not occurring for all of the bases of the respective strands but leaves a certain number or percentage of the bases unpaired or wrongly paired. The percentage of correctly pairing bases is preferably at least 70 %, more preferably 80 %, even more preferably 90 % and most preferably any percentage higher than 90 %. It is to be noted that a percentage of 70 % matching bases is considered as homology and the hybridization having this extent of matching base pairs is considered as stringent. Hybridization conditions for this kind of stringent hybridization may be taken from Current Protocols in Molecular Biology (John Wiley and Sons, Inc., 1987). More particularly, the hybridization conditions can be as follows:

- Hybridization performed e.g. in 5 x SSPE, 5 x Denhardt's reagent, 0.1% SDS, 100 g/mL sheared DNA at 68°C
- Moderate stringency wash in 0.2xSSC, O.1% SDS at 42°C
- High stringency wash in 0.1xSSC, 0.1% SDS at 68°C

Genomic DNA with a GC content of 50% has an approximate T_M of 96°C. For 1% mismatch, the T_M is reduced by approximately 1°C.

In addition, any of the further hybridization conditions described herein are in principle applicable as well.

Of course, all nucleic acid sequence molecules which encode the same polypeptide molecule as those identified by the present invention are encompassed by any disclosure of a given coding sequence, since the degeneracy of the genetic code is directly applicable to unambiguously determine all possible nucleic acid molecules which encode a given polypeptide molecule, even if the number of such degenerated

nucleic acid molecules may be high. This is also applicable for fragments of a given polypeptide, as long as the fragments encode a polypeptide being suitable to be used in a vaccination connection, e.g. as an active or passive vaccine.

The nucleic acid molecule according to the present invention can as a third alternative also be a nucleic acid which comprises a stretch of at least 15 bases of the nucleic acid molecule according to the first and second alternative of the nucleic acid molecules according to the present invention as outlined above. Preferably, the bases form a contiguous stretch of bases. However, it is also within the scope of the present invention that the stretch consists of two or more moieties, which are separated by a number of bases.

The present nucleic acids may preferably consist of at least 20, even more preferred at least 30, especially at least 50 contiguous bases from the sequences disclosed herein. The suitable length may easily be optimized due to the planned area of use (e.g. as (PCR) primers, probes, capture molecules (e.g. on a (DNA) chip), etc.). Preferred nucleic acid molecules contain at least a contiguous 15 base portion of one or more of the predicted immunogenic amino acid sequences listed in tables 1 and 2, especially the sequences of table 2 with scores of more than 10, preferably more than 20, especially with a score of more than 25. Specifically preferred are nucleic acids containing a contiguous portion of a DNA sequence of any sequence in the sequence protocol of the present application which shows 1 or more, preferably more than 2, especially more than 5, non-identical nucleic acid residues compared to the published *Streptococcus agalactiae* strain NEM316 (ATCC 12403) genome ({Glaser, P. et al., 2002}; GenBank accession AL732656) and/or any other published *S. agalactiae* genome sequence or parts thereof, especially of the serotype V 2603 V/R (A909) strain {Tettelin, H. et al., 2002}); GenBank accession AE009948). Specifically preferred non-identical nucleic acid residues are residues, which lead to a non-identical amino acid residue. Preferably, the nucleic acid sequences encode polypeptides having at least 1, preferably at least 2, preferably at least 3 different amino acid residues compared to the published *S. agalactiae* counterparts mentioned above. Also such isolated polypeptides, being fragments of the proteins (or the whole protein) mentioned herein e.g. in the sequence listing, having at least 6, 7, or 8 amino acid residues and being encoded by these nucleic acids are preferred.

The nucleic acid molecule according to the present invention can as a fourth alternative also be a nucleic acid molecule which anneals under stringent hybridisation conditions to any of the nucleic acids of the present invention according to the above outlined first, second, and third alternative. Stringent hybridisation conditions are typically those described herein.

Finally, the nucleic acid molecule according to the present invention can as a fifth alternative also be a nucleic acid molecule which, but for the degeneracy of the genetic code, would hybridise to any of the nucleic acid molecules according to any nucleic acid molecule of the present invention according to the first, second, third, and fourth alternative as outlined above. This kind of nucleic acid molecule refers to the fact that preferably the nucleic acids according to the present invention code for the hyperimmune serum reactive antigens or fragments thereof according to the present invention. This kind of nucleic acid molecule is particularly useful in the detection of a nucleic acid molecule according to the present invention and thus the diagnosis of the respective microorganisms such as *S. agalactiae* and any disease or diseased condition where this kind of microorganims is involved. Preferably, the hybridisation would occur or be preformed under stringent conditions as described in connection with the fourth alternative described above.

Nucleic acid molecule as used herein generally refers to any ribonucleic acid molecule or deoxyribonucleic acid molecule, which may be unmodified RNA or DNA or modified RNA or DNA. Thus, for instance, nucleic acid molecule as used herein refers to, among other, single-and double-stranded DNA, DNA that is a mixture of single- and double-stranded RNA, and RNA that is a mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-

stranded or, more typically, double-stranded, or triple-stranded, or a mixture of single- and double-stranded regions. In addition, nucleic acid molecule as used herein refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The strands in such regions may be from the same molecule or from different molecules. The regions may include all of one or more of the molecules, but more typically involve only a region of some of the molecules. One of the molecules of a triple-helical region often is an oligonucleotide. As used herein, the term nucleic acid molecule includes DNAs or RNAs as described above that contain one or more modified bases. Thus, DNAs or RNAs with backbones modified for stability or for other reasons are "nucleic acid molecule" as that term is intended herein. Moreover, DNAs or RNAs comprising unusual bases, such as inosine, or modified bases, such as tritylated bases, to name just two examples, are nucleic acid molecule as the term is used herein. It will be appreciated that a great variety of modifications have been made to DNA and RNA that serve many useful purposes known to those of skill in the art. The term nucleic acid molecule as it is employed herein embraces such chemically, enzymatically or metabolically modified forms of nucleic acid molecule, as well as the chemical forms of DNA and RNA characteristic of viruses and cells, including simple and complex cells, *inter alia.* The term nucleic acid molecule also embraces short nucleic acid molecules often referred to as oligonucleotide(s). "Polynucleotide" and "nucleic acid" or "nucleic acid molecule" are often used interchangeably herein.

Nucleic acid molecules provided in the present invention also encompass numerous unique fragments, both longer and shorter than the nucleic acid molecule sequences set forth in the sequencing listing of the *S. agalactiae* coding regions, which can be generated by standard cloning methods. To be unique, a fragment must be of sufficient size to distinguish it from other known nucleic acid sequences, most readily determined by comparing any selected *S. agalactiae* fragment to the nucleotide sequences in computer databases such as GenBank.

Additionally, modifications can be made to the nucleic acid molecules and polypeptides that are encompassed by the present invention. For example, nucleotide substitutions can be made which do not affect the polypeptide encoded by the nucleic acid, and thus any nucleic acid molecule which encodes a hyperimmune serum reactive antigen or fragments thereof is encompassed by the present invention.

Furthermore, any of the nucleic acid molecules encoding hyperimmune serum reactive antigens or fragments thereof provided by the present invention can be functionally linked, using standard techniques such as standard cloning techniques, to any desired regulatory sequences, whether a *S. agalactiae* regulatory sequence or a heterologous regulatory sequence, heterologous leader sequence, heterologous marker sequence or a heterologous coding sequence to create a fusion protein.

Nucleic acid molecules of the present invention may be in the form of RNA, such as mRNA or cRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced by chemical synthetic techniques or by a combination thereof. The DNA may be triple-stranded, double-stranded or single-stranded. Single-stranded DNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

The present invention further relates to variants of the herein above described nucleic acid molecules which encode fragments, analogs and derivatives of the hyperimmune serum reactive antigens and fragments thereof having a deducted *S. agalactiae* amino acid sequence set forth in the Sequence Listing. A variant of the nucleic acid molecule may be a naturally occurring variant such as a naturally occurring allelic variant, or it may be a variant that is not known to occur naturally. Such non-naturally occurring variants of the nucleic acid molecule may be made by mutagenesis techniques, including those applied to nucleic acid molecules, cells or organisms.

Among variants in this regard are variants that differ from the aforementioned nucleic acid molecules by nucleotide substitutions, deletions or additions. The substitutions, deletions or additions may involve one

or more nucleotides. The variants may be altered in coding or non-coding regions or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or additions. Preferred are nucleic acid molecules encoding a variant, analog, derivative or fragment, or a variant, analogue or derivative of a fragment, which have a *S. agalactiae* sequence as set forth in the Sequence Listing, in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid(s) is substituted, deleted or added, in any combination. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the *S. agalactiae* polypeptides set forth in the Sequence Listing. Also especially preferred in this regard are conservative substitutions.

The peptides and fragments according to the present invention also include modified epitopes wherein preferably one or two of the amino acids of a given epitope are modified or replaced according to the rules disclosed in e.g. {Tourdot, S. et al., 2000}, as well as the nucleic acid sequences encoding such modified epitopes.

It is clear that also epitopes derived from the present epitopes by amino acid exchanges improving, conserving or at least not significantly impeding the T cell activating capability of the epitopes are covered by the epitopes according to the present invention. Therefore the present epitopes also cover epitopes, which do not contain the original sequence as derived from *S. agalactiae*, but trigger the same or preferably an improved T cell response. These epitope are referred to as "heteroclitic"; they need to have a similar or preferably greater affinity to MHC/HLA molecules, and the need the ability to stimulate the T cell receptors (TCR) directed to the original epitope in a similar or preferably stronger manner.

Heteroclitic epitopes can be obtained by rational design i.e. taking into account the contribution of individual residues to binding to MHC/HLA as for instance described by {Rammensee, H. et al., 1999}, combined with a systematic exchange of residues potentially interacting with the TCR and testing the resulting sequences with T cells directed against the original epitope. Such a design is possible for a skilled man in the art without much experimentation.

Another possibility includes the screening of peptide libraries with T cells directed against the original epitope. A preferred way is the positional scanning of synthetic peptide libraries. Such approaches have been described in detail for instance by {Hemmer, B. et al., 1999}and the references given therein.

As an alternative to epitopes represented by the present derived amino acid sequences or heteroclitic epitopes, also substances mimicking these epitopes e.g. "peptidemimetica" or "retro-inverso-peptides" can be applied.

Another aspect of the design of improved epitopes is their formulation or modification with substances increasing their capacity to stimulate T cells. These include T helper cell epitopes, lipids or liposomes or preferred modifications as described in WO 01/78767.

Another way to increase the T cell stimulating capacity of epitopes is their formulation with immune stimulating substances for instance cytokines or chemokines like interleukin-2, -7, -12, -18, class I and II interferons (IFN), especially IFN-gamma, GM-CSF, TNF-alpha, flt3-ligand and others.

As discussed additionally herein regarding nucleic acid molecule assays of the invention, for instance, nucleic acid molecules of the invention as discussed above, may be used as a hybridization probe for RNA, cDNA and genomic DNA to isolate full-length cDNAs and genomic clones encoding polypeptides of the present invention and to isolate cDNA and genomic clones of other genes that have a high sequence similarity to the nucleic acid molecules of the present invention. Such probes generally will comprise at least 15 bases. Preferably, such probes will have at least 20, at least 25 or at least 30 bases, and may have at least 50 bases. Particularly preferred probes will have at least 30 bases, and will have 50 bases or less, such as 30, 35, 40, 45, or 50 bases.

For example, the coding region of a nucleic acid molecule of the present invention may be isolated by screening a relevant library using the known DNA sequence to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the present invention is then used to screen a library of cDNA, genomic DNA or mRNA to determine to which members of the library the probe hybridizes.

The nucleic acid molecules and polypeptides of the present invention may be employed as reagents and materials for development of treatments of and diagnostics for disease, particularly human disease, as further discussed herein relating to nucleic acid molecule assays, *inter alia*.

The nucleic acid molecules of the present invention that are oligonucleotides can be used in the processes herein as described, but preferably for PCR, to determine whether or not the *S. agalactiae* genes identified herein in whole or in part are present and/or transcribed in infected tissue such as blood. It is recognized that such sequences will also have utility in diagnosis of the stage of infection and type of infection the pathogen has attained. For this and other purposes the arrays comprising at least one of the nucleic acids according to the present invention as described herein, may be used.

The nucleic acid molecules according to the present invention may be used for the detection of nucleic acid molecules and organisms or samples containing these nucleic acids. Preferably such detection is for diagnosis, more preferable for the diagnosis of a disease related or linked to the present or abundance of *S. agalactiae.*

Eukaryotes (herein also "individual(s)"), particularly mammals, and especially humans, infected with *S. agalactiae* may be identifiable by detecting any of the nucleic acid molecules according to the present invention detected at the DNA level by a variety of techniques. Preferred nucleic acid molecules candidates for distinguishing a *S. agalactiae* from other organisms can be obtained.

The invention provides a process for diagnosing disease, arising from infection with *S. agalactiae*, comprising determining from a sample isolated or derived from an individual an increased level of expression of a nucleic acid molecule having the sequence of a nucleic acid molecule set forth in the Sequence Listing. Expression of nucleic acid molecules can be measured using any one of the methods well known in the art for the quantitation of nucleic acid molecules, such as, for example, PCR, RT-PCR, Rnase protection, Northern blotting, other hybridisation methods and the arrays described herein.

Isolated as used herein means separated "by the hand of man" from its natural state; i.e., that, if it occurs in nature, it has been changed or removed from its original environment, or both. For example, a naturally occurring nucleic acid molecule or a polypeptide naturally present in a living organism in its natural state is not "isolated," but the same nucleic acid molecule or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein. As part of or following isolation, such nucleic acid molecules can be joined to other nucleic acid molecules, such as DNAs, for mutagenesis, to form fusion proteins, and for propagation or expression in a host, for instance. The isolated nucleic acid molecules, alone or joined to other nucleic acid molecules such as vectors, can be introduced into host cells, in culture or in whole organisms. Introduced into host cells in culture or in whole organisms, such DNAs still would be isolated, as the term is used herein, because they would not be in their naturally occurring form or environment. Similarly, the nucleic acid molecules and polypeptides may occur in a composition, such as a media formulations, solutions for introduction of nucleic acid molecules or polypeptides, for example, into cells, compositions or solutions for chemical or enzymatic reactions, for instance, which are not naturally occurring compositions, and, therein remain isolated nucleic acid molecules or polypeptides within the meaning of that term as it is employed herein.

The nucleic acids according to the present invention may be chemically synthesized. Alternatively, the

nucleic acids can be isolated from *S. agalactiae* by methods known to the one skilled in the art.

According to another aspect of the present invention, a comprehensive set of novel hyperimmune serum reactive antigens and fragments thereof are provided by using the herein described antigen identification method. In a preferred embodiment of the invention, a hyperimmune serum-reactive antigen comprising an amino acid sequence being encoded by any one of the nucleic acids molecules herein described and fragments thereof are provided. In another preferred embodiment of the invention a novel set of hyperimmune serum-reactive antigens which comprises amino acid sequences selected from a group consisting of the polypeptide sequences as represented in **Seq ID No** 231, 307, 374-433 and fragments thereof are provided. In a further preferred embodiment of the invention hyperimmune serum-reactive antigens which comprise amino acid sequences selected from a group consisting of the polypeptide sequences as represented in **Seq ID No** 218, 220, 222-230, 232, 235-242, 244-248, 250-253, 256-285, 287-302, 309-317, 320-343, 345-362, 364, 366-373, 434, 449-462 and 475-486 and fragments thereof are provided. In a still preferred embodiment of the invention hyperimmune serum-reactive antigens which comprise amino acid sequences selected from a group consisting of the polypeptide sequences as represented in **Seq ID No** 249, 303, 308, 318, 344 and fragments thereof are provided.

The hyperimmune serum reactive antigens and fragments thereof as provided in the invention include any polypeptide set forth in the Sequence Listing as well as polypeptides which have at least 70% identity to a polypeptide set forth in the Sequence Listing, preferably at least 80% or 85% identity to a polypeptide set forth in the Sequence Listing, and more preferably at least 90% similarity (more preferably at least 90% identity) to a polypeptide set forth in the Sequence Listing and still more preferably at least 95%, 96%, 97%, 98%, 99% or 99.5% similarity (still more preferably at least 95%, 96%, 97%, 98%, 99%, or 99.5% identity) to a polypeptide set forth in the Sequence Listing and also include portions of such polypeptides with such portion of the polypeptide generally containing at least 4 amino acids and more preferably at least 8, still more preferably at least 30, still more preferably at least 50 amino acids, such as 4, 8, 10, 20, 30, 35, 40, 45 or 50 amino acids.

The invention also relates to fragments, analogs, and derivatives of these hyperimmune serum reactive antigens and fragments thereof. The terms "fragment", "derivative" and "analog" when referring to an antigen whose amino acid sequence is set forth in the Sequence Listing, means a polypeptide which retains essentially the same or a similar biological function or activity as such hyperimmune serum reactive antigen and fragment thereof.

The fragment, derivative or analog of a hyperimmune serum reactive antigen and fragment thereof may be 1) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or 2) one in which one or more of the amino acid residues includes a substituent group, or 3) one in which the mature hyperimmune serum reactive antigen or fragment thereof is fused with another compound, such as a compound to increase the half-life of the hyperimmune serum reactive antigen and fragment thereof (for example, polyethylene glycol), or 4) one in which the additional amino acids are fused to the mature hyperimmune serum reactive antigen or fragment thereof, such as a leader or secretory sequence or a sequence which is employed for purification of the mature hyperimmune serum reactive antigen or fragment thereof or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

The present invention also relates to antigens of different *S. agalactiae* isolates. Such homologues may easily be isolated based on the nucleic acid and amino acid sequences disclosed herein. There are 9 serotypes distinguished to date and the typing is based on serotype specific antisera. The presence of any antigen can accordingly be determined for every serotype. In addition it is possible to determine the variability of a particular antigen in the various serotypes as described for the *S. pyogenes* sic gene {Hoe,

N. et al., 2001}. The contribution of the various serotypes to the different GBS infections varies in the different age groups and geographical regions. It is an important aspect that the most valuable protective antigens are expected to be conserved among various clinical strains.

Among the particularly preferred embodiments of the invention in this regard are the hyperimmune serum reactive antigens set forth in the Sequence Listing, variants, analogs, derivatives and fragments thereof, and variants, analogs and derivatives of fragments. Additionally, fusion polypeptides comprising such hyperimmune serum reactive antigens, variants, analogs, derivatives and fragments thereof, and variants, analogs and derivatives of the fragments are also encompassed by the present invention. Such fusion polypeptides and proteins, as well as nucleic acid molecules encoding them, can readily be made using standard techniques, including standard recombinant techniques for producing and expression of a recombinant polynucleic acid encoding a fusion protein.

Among preferred variants are those that vary from a reference by conservative amino acid substitutions. Such substitutions are those that substitute a given amino acid in a polypeptide by another amino acid of like characteristics. Typically seen as conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu and Ile; interchange of the hydroxyl residues Ser and Thr, exchange of the acidic residues Asp and Glu, substitution between the amide residues Asn and Gln, exchange of the basic residues Lys and Arg and replacements among the aromatic residues Phe and Tyr.

Further particularly preferred in this regard are variants, analogs, derivatives and fragments, and variants, analogs and derivatives of the fragments, having the amino acid sequence of any polypeptide set forth in the Sequence Listing, in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, deleted or added, in any combination. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the polypeptide of the present invention. Also especially preferred in this regard are conservative substitutions. Most highly preferred are polypeptides having an amino acid sequence set forth in the Sequence Listing without substitutions.

The hyperimmune serum reactive antigens and fragments thereof of the present invention are preferably provided in an isolated form, and preferably are purified to homogeneity.

Also among preferred embodiments of the present invention are polypeptides comprising fragments of the polypeptides having the amino acid sequence set forth in the Sequence Listing, and fragments of variants and derivatives of the polypeptides set forth in the Sequence Listing.

In this regard a fragment is a polypeptide having an amino acid sequence that entirely is the same as part but not all of the amino acid sequence of the afore mentioned hyperimmune serum reactive antigen and fragment thereof, and variants or derivative, analogs, fragments thereof. Such fragments may be "free-standing", i.e., not part of or fused to other amino acids or polypeptides, or they may be comprised within a larger polypeptide of which they form a part or region. Also preferred in this aspect of the invention are fragments characterised by structural or functional attributes of the polypeptide of the present invention, i.e. fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta-amphipathic regions, flexible regions, surface-forming regions, substrate binding regions, and high antigenic index regions of the polypeptide of the present invention, and combinations of such fragments. Preferred regions are those that mediate activities of the hyperimmune serum reactive antigens and fragments thereof of the present invention. Most highly preferred in this regard are fragments that have a chemical, biological or other activity of the hyperimmune serum reactive antigen and fragments thereof of the present invention, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Particularly preferred are fragments comprising receptors or domains of enzymes that confer a function essential for

viability of *S. agalactiae* or the ability to cause disease in humans. Further preferred polypeptide fragments are those that comprise or contain antigenic or immunogenic determinants in an animal, especially in a human.

An antigenic fragment is defined as a fragment of the identified antigen, which is for itself antigenic or may be made antigenic when provided as a hapten. Therefore, also antigens or antigenic fragments showing one or (for longer fragments) only a few amino acid exchanges are enabled with the present invention, provided that the antigenic capacities of such fragments with amino acid exchanges are not severely deteriorated on the exchange(s), i.e., suited for eliciting an appropriate immune response in an individual vaccinated with this antigen and identified by individual antibody preparations from individual sera.

Preferred examples of such fragments of hyperimmune serum-reactive antigens selected from the group consisting of peptides comprising amino acid sequences of column "predicted immunogenic aa" and "location of identified immunogenic region" of Table 1**A, especially peptides comprising amino acid** 4-20, 35-44, 65-70, 73-87, 92-98, 112-137, 152-161, 177-186, 193-200, 206-213, 229-255, 282-294, 308-313, 320-326, 349-355, 373-384, 388-406, 420-425 and 115-199 of **Seq ID No** 218; 5-24, 35-41, 44-70, 73-89, 103-109, 127-143, 155-161, 185-190, 192-207, 212-219, 246-262, 304-336, 372-382, 384-393, 398-407, 412-418, 438-444, 1-75, 76-161 and 164-239 of **Seq ID No** 219; 4-10, 16-58, 60-71, 77-92, 100-126, 132-146, 149-164, 166-172, 190-209, 214-220, 223-229, 241-256, 297-312, 314-319, 337-343, 351-359, 378-387, 398-418, 421-428, 430-437, 440-448, 462-471, 510-519, 525-536, 552-559, 561-568, 573-582, 596-602, 608-630, 637-649, 651-665, 681-702, 714-732, 739-745, 757-778, 790-805, 807-815, 821-829, 836-842, 846-873, 880-903, 908-914, 916-923, 931-940, 943-948, 956-970, 975-986, 996-1015, 1031-1040, 1051-1069, 1072-1095, 1114-1119, 1130-1148, 1150-1157, 1169-1176, 1229-1238 and 802-812 of **Seq ID No** 220; 5-12, 14-26, 35-47, 52-67, 72-78, 83-98, 121-141, 152-159, 163-183, 186-207, 209-257, 264-277, 282-299, 301-309, 312-318, 324-339, 358-368, 372-378, 387-397, 425-431 and 46-291 of **Seq ID No** 221; 29-38, 44-64, 70-76, 78-87, 94-100, 102-112, 119-134, 140-149, 163-173, 178-186, 188-194, 207-234, 247-262, 269-290 and 73-92 of **Seq ID No** 222; 10-28, 36-63, 77-87, 103-119, 127-136, 141-169, 171-183, 195-200, 207-232, 236-246, 251-265, 268-283, 287-297, 314-322, 335-343, 354-363, 384-390, 405-411, 419-436, 443-455, 467-473, 480-513, 518-529, 550-557, 565-585, 602-608, 616-625, 632-660, 665-677, 685-701, 726-736, 738-747, 752-761, 785-796, 801-813, 838-853, 866-871 and 757-774 of **Seq ID No** 223; 31-38, 61-66, 74-81, 90-115, 123-145, 154-167, 169-179, 182-193, 200-206, 238-244, 267-272 and 235-251 of **Seq ID No** 224; 19-25, 38-54, 56-64, 66-72, 74-92, 94-100, 116-129, 143-149, 156-183, 204-232, 253-266, 269-275, 294-307 and 241-313 of **Seq ID No** 225; 5-34, 50-56, 60-65, 74-85, 89-97, 108-119, 159-165, 181-199, 209-225, 230-240, 245-251, 257-262, 274-282, 300-305 and 64-75 of **Seq ID No** 226; 5-13, 16-21, 27-42, 45-52, 58-66, 74-87, 108-114, 119-131 and 39-51 of **Seq ID No** 227; 6-23, 46-54, 59-65, 78-84, 100-120, 128-133, 140-146, 159-165, 171-183, 190-204, 224-232, 240-248, 250-259, 274-280, 288-296, 306-315 and 267-274 of **Seq ID No** 228; 5-12, 15-24, 26-36, 42-65, 68-80, 82-104, 111-116, 125-144, 159-167, 184-189, 209-218, 235-243, 254-265, 269-283, 287-300, 306-316, 318-336, 338-352, 374-392 and 162-174 of **Seq ID No** 229; 30-42, 45-54 and 25-37 of **Seq ID No** 230; 10-30, 53-59, 86-95, 116-130, 132-147, 169-189, 195-201, 212-221, 247-256, 258-265, 278-283, 291-298, 310-316, 329-339, 341-352, 360-367, 388-396, 398-411, 416-432, 443-452, 460-466, 506-512, 515-521, 542-548 and 419-431 of **Seq ID No** 231; 4-27, 30-53, 60-67, 70-90, 92-151, 159-185, 189-195, 198-210, 215-239 and 173-189 of **Seq ID No** 232; 4-26, 41-54, 71-78, 116-127, 140-149, 151-158, 161-175, 190-196, 201-208, 220-226, 240-252, 266-281, 298-305, 308-318, 321-329, 344-353, 372-378, 384-405, 418-426, 429-442, 457-463, 494-505, 514-522 and 174-188 of **Seq ID No** 233; 17-25, 27-39, 61-67, 81-89, 99-110, 120-131, 133-139, 147-161, 167-172, 179-185, 192-198, 203-213, 226-238, 243-258, 261-267, 284-290, 296-307, 311-328, 340-352, 356-371 and 239-256 of **Seq ID No** 234; 8-30, 40-49, 67-80, 114-123, 126-142, 152-162, 188-194 and 57-70 of **Seq ID No** 235; 4-23, 28-34, 36-47, 50-61, 76-81, 89-94, 96-104, 112-119, 126-146, 155-181, 195-200, 208-214, 220-229, 244-260, 263-276, 282-288, 292-300, 317-323, 336-351, 353-359, 363-375, 382-399, 415-432, 444-455, 458-471, 476-481, 484-492, 499-517, 522-529, 535-541, 543-568, 572-584, 586-600, 607-617, 626-637, 656-675 and 282-297 of **Seq ID No** 236; 6-24, 30-35, 38-45, 63-91, 134-140, 146-160, 167-188, 214-220, 226-234, 244-250, 260-270, 286-301, 316-329, 340-371, 429-446, 448-459, 474-481, 485-491, 512-526, 537-544, 550-565, 573-583, 596-613, 621-630, 652-658 and 87-97 of **Seq ID No** 237; 8-20, 26-48, 56-67, 76-86, 94-109, 115-121, 123-

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12-28, 41-91, 98-107, 112-120, 125-131, 151-193, 215-221, 240-250, 263-280 and 128-138 of **Seq ID No** 368; 16-24, 32-38, 46-62, 68-81, 90-105, 127-133, 144-150, 160-166, 178-184, 186-202, 210-219, 232-240, 252-258, 264-273, 293-324, 337-344, 349-357, 360-369,

385-398, 410-416, 419-427, 441-449, 458-476, 508-515, 523-539, 544-549, 562-569, 571-579, 96-109 and 127-139 of **Seq ID No** 369; 19-25, 28-34, 56-61, 85-97, 110-116 and 39-53 of **Seq ID No** 370; 4-37, 41-50, 62-72, 91-97, 99-109, 114-125, 136-141, 149-158, 160-166, 201-215 and 27-225 of **Seq ID No** 371; 15-31, 44-51, 96-105, 122-130, 149-157, 162-168, 178-183, 185-192, 198-204, 206-213, 221-234, 239-245, 248-255, 257-266, 289-335, 349-357, 415-422, 425-441, 448-454, 462-468 and 463-481 of **Seq ID No** 372; 5-31, 39-55, 63-72, 76-99, 106-155, 160-177, 179-199, 207-217, 223-240, 245-255, 261-267, 294-316, 321-343, 354-378, 382-452, 477-488, 529-536, 555-569, 584-591, 593-612, 620-627, 632-640, 647-654, 671-680, 698-704, 723-730, 732-750, 769-775, 781-788, 822-852 and 505-525 of **Seq ID No** 373; 3-18 of **Seq ID No** 374; 4-14 and 12-24 of **Seq ID No** 375; 4-11, 22-30 and 12-25 of **Seq ID No** 376; 5-12 and 4-18 of **Seq ID No** 377; 4-28 and 7-14 of **Seq ID No** 378; 6-16 and 8-16 of **Seq ID No** 379; 4-15, 18-33 and 24-36 of **Seq ID No** 380; 4-10, 16-21 and 20-31 of **Seq ID No** 381; 6-19 of **Seq ID No** 382; 11-18 and 3-10 of **Seq ID No** 383; 13-24 and 3-15 of **Seq ID No** 384; 15-27 and 7-16 of **Seq ID No** 385; 11-16 and 1-15 of **Seq ID No** 386; 4-16 and 9-21 of **Seq ID No** 387; 4-24, 40-48, 54-67 and 22-39 of **Seq ID No** 388; 6-30, 34-55, 62-68, 78-106 and 68-74 of **Seq ID No** 389; 3-14 of **Seq ID No** 390; 9-19 and 6-21 of **Seq ID No** 391; 4-17 and 1-9 of **Seq ID No** 392; 5-30 and 1-8 of **Seq ID No** 393; 4-16, 23-46, 51-56 and 45-55 of **Seq ID No** 394; 7-16 of **Seq ID No** 395; 2-14 of **Seq ID No** 396; 4-36, 43-65 and 50-62 of **Seq ID No** 397; 10-30 and 14-21 of **Seq ID No** 398; 9-17 and 1-10 of **Seq ID No** 399; 4-12 and 3-16 of **Seq ID No** 400; 4-15 and 5-23 of **Seq ID No** 401; 10-21 of **Seq ID No** 402; 6-16 of **Seq ID No** 403; 4-29, 31-38 and 2-14 of **Seq ID No** 404; 4-35 and 33-42 of **Seq ID No** 405; 2-17 of **Seq ID No** 406; 9-18, 30-35 and 15-33 of **Seq ID No** 407; 4-9 and 6-12 of **Seq ID No** 408; 3-17 of **Seq ID No** 409; 12-21, 37-44, 52-61, 72-80 and 38-48 of **Seq ID No** 410; 4-10, 29-44, 54-61, 69-78 and 13-27 of **Seq ID No** 411; 13-23, 36-53 and 2-15 of **Seq ID No** 412; 4-25, 28-46, 56-72, 81-99, 120-132, 134-142, 154-160 and 129-141 of **Seq ID No** 413; 4-15, 24-33, 35-41, 64-86 and 21-33 of **Seq ID No** 414; 9-15 and 4-13 of **Seq ID No** 415; 4-11, 13-19, 34-48 and 15-32 of **Seq ID No** 416; 4-21 and 11-31 of **Seq ID No** 417; 23-57 and 38-50 of **Seq ID No** 418; 4-32 and 3-13 of **Seq ID No** 419; 4-10, 13-25, 32-42, 56-68, 72-84 and 26-38 of **Seq ID No** 420; 4-20, 31-48, 52-58, 65-71, 80-93, 99-108, 114-123 and 37-49 of **Seq ID No** 421; 6-12, 14-20 and 3-25 of **Seq ID No** 422; 14-25, 27-38 and 5-14 of **Seq ID No** 423; 4-41, 57-105, 109-118, 123-136, 144-152 and 86-99 of **Seq ID No** 424; 6-19 of **Seq ID No** 425; 2-19 of **Seq ID No** 426; 14-47 and 1-14 of **Seq ID No** 427; 4-21, 29-44 and 2-18 of **Seq ID No** 428; 23-29 and 10-28 of **Seq ID No** 429; 6-16, 22-36 and 11-22 of **Seq ID No** 430; 4-19, 30-44 and 18-27 of **Seq ID No** 431; 5-15, 37-45, 58-65 and 38-47 of **Seq ID No** 432; 4-15, 23-34 and 4-15 of **Seq ID No** 433; 30-36, 44-54, 79-85, 101-114, 138-152, 154-164, 170-175, 179-200, 213-220, 223-240, 243-255, 258-264, 268-284 and 10-28 of **Seq ID No** 434; **the peptides comprising amino acid sequences of column "Identical region" of the Table 1B, especially peptides comprising amino acid** 210-226 and 738-753 of **Seq ID No** 449; 326-344, 326-348, 338-354, 371-392, 801-809 and 877-901 of **Seq ID No** 450; 893-906 of **Seq ID No** 451; 51-69 of **Seq ID No** 452; 110-125 of **Seq ID No** 453; 291-305 of **Seq ID No** 454; 210-226 and 738-753 of **Seq ID No** 455; 326-344, 326-348, 338-354, 371-392, 801-809 and 877-901 of **Seq ID No** 456; 893-906 of **Seq ID No** 457; 51-69 of **Seq ID No** 458; 110-125 of **Seq ID No** 459; 291-305 of **Seq ID No** 460; 32-44 of **Seq ID No** 461; 399-410 of **Seq ID No** 462; the serum reactive epitopes **as specified in the column of "aa from" to "aa to"** of Table **2, especially peptides comprising amino acid** 120-143, 138-161 and 156-179 of **Seq ID No** 218; 110-129 and 168-184 of **Seq ID No** 219; 74-90 of **Seq ID No** 222; 759-773 of **Seq ID No** 223; 237-260 of **Seq ID No** 224; 265-284 of **Seq ID No** 225; 65-74 of **Seq ID No** 226; 41-50 of **Seq ID No** 227; 163-174 of **Seq ID No** 229; 26-37 of **Seq ID No** 230; 174-189 of **Seq ID No** 232; 240-256 of **Seq ID No** 234; 285-297 of **Seq ID No** 236; 238-247 of **Seq ID No** 238; 491-519 of **Seq ID No** 239; 114-140 of **Seq ID No** 243; 267-284 of **Seq ID No** 250; 439-453 of **Seq ID No** 252; 162-178 of **Seq ID No** 253; 347-364 of **Seq ID No** 254; 699-715 of **Seq ID No** 255; 60-71 of **Seq ID No** 256; 244-257 of **Seq ID No** 257; 44-63 and 57-76 of **Seq ID No** 258; 185-196 of **Seq ID No** 260; 119-129 of **Seq ID No** 263; 182-195 of **Seq ID No** 266; 32-44 and 424-442 of **Seq ID No** 267; 247-256 of **Seq ID No** 268; 678-694, 785-805, 55-77 and 72-94 of **Seq ID No** 269; 210-226 of **Seq ID No** 281; 37-59 of **Seq ID No** 289; 13-29 of **Seq ID No** 296; 136-159 of **Seq ID No** 348; 205-222 of **Seq ID No** 349; 99-110 of **Seq ID No** 350; 160-176 of **Seq ID No** 351; 457-470 of **Seq ID No** 355; 221-237 of **Seq ID No** 356; 167-190 of **Seq ID No** 357; 96-120 of **Seq ID No** 361; 399-417, 503-519 and 544-563 of **Seq ID No** 364; 46-68, 159-183 and 184-198 of **Seq ID No** 371; 463-481 of **Seq ID No** 372; **the immunogenic epitopes as specified in the column of "aa from" to "aa to" of Table 4;** especially peptides comprising amino acid 110-129 and 168-184 of **Seq ID No** 219; 877-901, 333-354, 326-344 and 801-809 of **Seq ID No** 277; 1-54 of

Seq ID No 347; 544-563, 31-51, 107-119, 399-417 and 503-519 of **Seq ID No** 364; 120-198 of **Seq ID No** 218; 20-35 of **Seq ID No** 219; 118-201 of **Seq ID No** 221; 48-132 of **Seq ID No** 242; 118-136 of **Seq ID No** 249; 162-178 of **Seq ID No** 253; 347-364 of **Seq ID No** 254; 699-715 of **Seq ID No** 255; 50-76 of **Seq ID No** 258; 785-819 and 44-128 of **Seq ID No** 269; 90-128 of **Seq ID No** 274; 314-384 of **Seq ID No** 289; 327-349 of **Seq ID No** 293; 242-314, 405-478 and 23-100 of **Seq ID No** 304; 129-210 of **Seq ID No** 305; 162-188 of **Seq ID No** 307; 750-772 of **Seq ID No** 310; 1-56 of **Seq ID No** 335; 322-337 of **Seq ID No** 337; 72-90 of **Seq ID No** 339; 374-395 of **Seq ID No** 345; 136-159 of **Seq ID No** 348; 141-164 of **Seq ID No** 358; 96-157 of **Seq ID No** 361; 1-82 of **Seq ID No** 363; 489-556 of **Seq ID No** 364; 159-183 and 49-133 of **Seq ID No** 371; **The peptides comprising amino acid sequences of column "predicted immunogenic aa" and "location of identified immunogenic region (aa)" of Table 5, especially peptides comprising amino acid** 4-26, 35-41, 53-61, 73-84, 103-108, 114-120, 140-146, 156-162, 192-208, 214-219, 227-233, 239-252, 260-268, 284-297, 1-48 and 113-133 of **Seq ID No** 475; 4-27, 38-44, 50-56, 59-64, 72-79, 83-89, 92-97, 108-116, 123-148, 152-167, 183-196, 200-220, 232-244, 255-261, 265-274, 282-302, 309-317, 1-79 and 231-302 of **Seq ID No** 476; 6-28, 66-72, 85-105, 115-121, 144-151, 160-170, 176-185, 223-230, 252-288, 296-310, 319-333, 367-374, 458-464, 471-480, 483-488, 520-528, 530-549, 559-564, 593-601, 606-616, 636-643, 655-662, 676-682, 684-699, 719-726, 735-750, 757-764, 777-785, 799-810, 812-843, 846-853, 868-873, 880-889, 891-899, 909-929, 934-940, 963-969, 998-1004, 1007-1014, 1016-1022, 1030-1046, 1-80 and 808-821 of **Seq ID No** 477; 7-24, 35-41, 75-81, 91-114, 122-132, 137-144, 148-156, 183-192, 194-200, 212-228, 233-238, 251-258, 275-295, 326-332, 337-346, 1-79 and 305-321 of **Seq ID No** 478; 31-38, 42-52, 66-72, 86-92, 98-104, 115-122, 127-146, 154-164, 169-187, 198-212, 225-237, 255-269, 13-92 and 135-142 of **Seq ID No** 479; 4-36, 39-49, 63-69, 71-77, 81-88, 123-131, 133-139, 160-169, 174-180, 188-194, 210-217, 273-278, 289-300, 317-334, 336-341, 383-401, 425-438, 1-68, 212-270 and 402-446 of **Seq ID No** 480; 21-29, 31-42, 49-63, 72-79, 81-93, 112-132, 159-165, 188-195, 197-232, 262-267, 279-286, 294-301, 318-326, 348-366, 381-405, 409-426, 436-465, 471-480, 484-492, 497-505, 521-544, 554-561, 567-577, 581-589, 601-609, 611-622, 636-651, 653-667, 669-685, 700-708, 716-722, 729-744, 749-766, 780-786, 789-811, 814-864, 1-57 and 84-106 of **Seq ID No** 481; 6-24, 35-48, 57-63, 72-78, 87-92, 113-119, 123-137, 147-153, 173-181, 212-233 and 1-124 of **Seq ID No** 482; 13-34, 62-69, 78-83, 86-91, 98-104, 107-115, 146-159, 179-188, 195-205, 209-221, 226-233, 239-253, 276-282, 284-294, 297-308, 331-354, 375-382, 388-399, 421-433, 449-458, 464-469, 472-491, 508-513, 525-531, 534-550, 575-593, 601-618, 629-635, 654-661, 666-680, 706-721, 723-740, 771-805, 810-830, 845-851 and 1-84 of **Seq ID No** 483; 4-32, 45-64, 73-83, 86-92, 100-111, 125-147, 157-163, 170-175, 177-188, 226-232, 245-252, 258-274, 320-335, 348-359 and 1-71 of **Seq ID No** 484; 13-40, 43-71, 76-83, 87-101, 109-119, 125-156, 162-175, 182-219, 226-232, 240-262, 270-287, 306-318, 326-342, 344-408, 414-444, 449-456 and 1-51 of **Seq ID No** 485; 4-16, 18-34, 45-54, 99-108, 134-140, 203-212, 241-257, 266-274, 279-291, 308-315, 330-336, 355-370, 374-382, 402-410, 428-455, 466-472, 474-480, 531-554, 560-566, 572-580, 597-618, 632-660, 664-674, 676-685, 691-705, 708-735, 750-768, 1-87 and 342-480 of **Seq ID No** 486; **The serum reactive epitopes as specified in the column of "aa from" to "aa to" of Table 6, especially peptides comprising amino acid** 115-132 and 1-26 of **Seq ID No** 475; 33-55 of **Seq ID No** 476; 1-25 of **Seq ID No** 478; 37-61 of **Seq ID No** 479; 1-24 of **Seq ID No** 480; 1-23 of **Seq ID No** 481; 46-60 of **Seq ID No** 482; 1-28, 23-50 and 45-71 of **Seq ID No** 483; 1-22 and 17-38 of **Seq ID No** 484; 1-22 and 17-38 of **Seq ID No** 485; 1-27, 22-47 and 422-447 of **Seq ID No** 486; **The immunogenic epitopes as specified in the column of "aa from" to "aa to" of Table 7, especially peptides comprising amino acid** 115-132 and 1-47 of **Seq ID No** 475; 1-55 of **Seq ID No** 476; 22-85 of **Seq ID No** 477; 307-320 and 1-44 of **Seq ID No** 478; 15-76 and 40-92 of **Seq ID No** 479; 1-59, 213-269 and 403-445 of **Seq ID No** 480; 1-56 and 85-105 of **Seq ID No** 481; 37-121 of **Seq ID No** 482; 1-71 of **Seq ID No** 483; 1-38 of **Seq ID No** 484; 1-38 of **Seq ID No** 485; 1-47 of **Seq ID No** 486 and fragments comprising at least 6, preferably more than 8, especially more than 10 aa and preferably not more than 70, 50, 40, 20, 15 or 11 aa of said sequences. All these fragments individually and each independently form a preferred selected aspect of the present invention.

All linear hyperimmune serum reactive fragments of a particular antigen may be identified by analysing the entire sequence of the protein antigen by a set of peptides overlapping by 1 amino acid with a length of at least 10 amino acids. Subsequently, non-linear epitopes can be identified by analysis of the protein antigen with hyperimmune sera using the expressed full-length protein or domain polypeptides thereof. Assuming that a distinct domain of a protein is sufficient to form the 3D structure independent from the

native protein, the analysis of the respective recombinant or synthetically produced domain polypeptide with hyperimmune serum would allow the identification of conformational epitopes within the individual domains of multi-domain proteins. For those antigens where a domain possesses linear as well as conformational epitopes, competition experiments with peptides corresponding to the linear epitopes may be used to confirm the presence of conformational epitopes.

It will be appreciated that the invention also relates to, among others, nucleic acid molecules encoding the aforementioned fragments, nucleic acid molecules that hybridise to nucleic acid molecules encoding the fragments, particularly those that hybridise under stringent conditions, and nucleic acid molecules, such as PCR primers, for amplifying nucleic acid molecules that encode the fragments. In these regards, preferred nucleic acid molecules are those that correspond to the preferred fragments, as discussed above.

The present invention also relates to vectors, which comprise a nucleic acid molecule or nucleic acid molecules of the present invention, host cells which are genetically engineered with vectors of the invention and the production of hyperimmune serum reactive antigens and fragments thereof by recombinant techniques.

A great variety of expression vectors can be used to express a hyperimmune serum reactive antigen or fragment thereof according to the present invention. Generally, any vector suitable to maintain, propagate or express nucleic acids to express a polypeptide in a host may be used for expression in this regard. In accordance with this aspect of the invention the vector may be, for example, a plasmid vector, a single or double-stranded phage vector, a single or double-stranded RNA or DNA viral vector. Starting plasmids disclosed herein are either commercially available, publicly available, or can be constructed from available plasmids by routine application of well-known, published procedures. Preferred among vectors, in certain respects, are those for expression of nucleic acid molecules and hyperimmune serum reactive antigens or fragments thereof of the present invention. Nucleic acid constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, the hyperimmune serum reactive antigens and fragments thereof of the invention can be synthetically produced by conventional peptide synthesizers. Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA construct of the present invention.

Host cells can be genetically engineered to incorporate nucleic acid molecules and express nucleic acid molecules of the present invention. Representative examples of appropriate hosts include bacterial cells, such as streptococci, staphylococci, *E. coli*, *Streptomyces* and *Bacillus subtillis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS, Hela, C127, 3T3, BHK, 293 and Bowes melanoma cells; and plant cells.

The invention also provides a process for producing a *S. agalactiae* hyperimmune serum reactive antigen and a fragment thereof comprising expressing from the host cell a hyperimmune serum reactive antigen or fragment thereof encoded by the nucleic acid molecules provided by the present invention. The invention further provides a process for producing a cell, which expresses a *S. agalactiae* hyperimmune serum reactive antigen or a fragment thereof comprising transforming or transfecting a suitable host cell with the vector according to the present invention such that the transformed or transfected cell expresses the polypeptide encoded by the nucleic acid contained in the vector.

The polypeptide may be expressed in a modified form, such as a fusion protein, and may include not only secretion signals but also additional heterologous functional regions. Thus, for instance, a region of additional amino acids, particularly charged amino acids, may be added to the N- or C-terminus of the polypeptide to improve stability and persistence in the host cell, during purification or during

subsequent handling and storage. Also, regions may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability or to facilitate purification, among others, are familiar and routine techniques in the art. A preferred fusion protein comprises a heterologous region from immunoglobulin that is useful to solubilize or purify polypeptides. For example, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobin molecules together with another protein or part thereof. In drug discovery, for example, proteins have been fused with antibody Fc portions for the purpose of high-throughout screening assays to identify antagonists. See for example, {Bennett, D. et al., 1995} and {Johanson, K. et al., 1995}.

The *S. agalactiae* hyperimmune serum reactive antigen or a fragment thereof can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, hydroxylapatite chromatography and lectin chromatography.

The hyperimmune serum reactive antigens and fragments thereof according to the present invention can be produced by chemical synthesis as well as by biotechnological means. The latter comprise the transfection or transformation of a host cell with a vector containing a nucleic acid according to the present invention and the cultivation of the transfected or transformed host cell under conditions, which are known to the ones skilled in the art. The production method may also comprise a purification step in order to purify or isolate the polypeptide to be manufactured. In a preferred embodiment the vector is a vector according to the present invention.

The hyperimmune serum reactive antigens and fragments thereof according to the present invention may be used for the detection of the organism or organisms in a sample containing these organisms or polypeptides derived thereof. Preferably such detection is for diagnosis, more preferable for the diagnosis of a disease, most preferably for the diagnosis of a diseases related or linked to the presence or abundance of Gram-positive bacteria, especially bacteria selected from the group comprising streptococci, staphylococci and lactococci. More preferably, the microorganisms are selected from the group comprising *Streptococcus pneumoniae*, *Streptococcus pyogenes* and *Streptococcus mutans*, especially the microorganism is *Streptococcus pyogenes*.

The present invention also relates to diagnostic assays such as quantitative and diagnostic assays for detecting levels of the hyperimmune serum reactive antigens and fragments thereof of the present invention in cells and tissues, including determination of normal and abnormal levels. Thus, for instance, a diagnostic assay in accordance with the invention for detecting over-expression of the polypeptide compared to normal control tissue samples may be used to detect the presence of an infection, for example, and to identify the infecting organism. Assay techniques that can be used to determine levels of a polypeptide, in a sample derived from a host are well known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays. Among these, ELISAs frequently are preferred. An ELISA assay initially comprises preparing an antibody specific to the polypeptide, preferably a monoclonal antibody. In addition, a reporter antibody generally is prepared which binds to the monoclonal antibody. The reporter antibody is attached to a detectable reagent such as radioactive, fluorescent or enzymatic reagent, such as horseradish peroxidase enzyme.

The hyperimmune serum reactive antigens and fragments thereof according to the present invention may also be used for the purpose of or in connection with an array. More particularly, at least one of the hyperimmune serum reactive antigens and fragments thereof according to the present invention may be immobilized on a support. Said support typically comprises a variety of hyperimmune serum reactive

antigens and fragments thereof whereby the variety may be created by using one or several of the hyperimmune serum reactive antigens and fragments thereof according to the present invention and/or hyperimmune serum reactive antigens and fragments thereof being different. The characterizing feature of such array as well as of any array in general is the fact that at a distinct or predefined region or position on said support or a surface thereof, a distinct polypeptide is immobilized. Because of this any activity at a distinct position or region of an array can be correlated with a specific polypeptide. The number of different hyperimmune serum reactive antigens and fragments thereof immobilized on a support may range from as little as 10 to several 1000 different hyperimmune serum reactive antigens and fragments thereof. The density of hyperimmune serum reactive antigens and fragments thereof per cm^2 is in a preferred embodiment as little as 10 peptides/polypeptides per cm^2 to at least 400 different peptides/polypeptides per cm^2 and more particularly at least 1000 different hyperimmune serum reactive antigens and fragments thereof per cm^2.

The manufacture of such arrays is known to the one skilled in the art and, for example, described in US patent 5,744,309. The array preferably comprises a planar, porous or non-porous solid support having at least a first surface. The hyperimmune serum reactive antigens and fragments thereof as disclosed herein, are immobilized on said surface. Preferred support materials are, among others, glass or cellulose. It is also within the present invention that the array is used for any of the diagnostic applications described herein. Apart from the hyperimmune serum reactive antigens and fragments thereof according to the present invention also the nucleic acid molecules according to the present invention may be used for the generation of an array as described above. This applies as well to an array made of antibodies, preferably monoclonal antibodies as, among others, described herein.

In a further aspect the present invention relates to an antibody directed to any of the hyperimmune serum reactive antigens and fragments thereof, derivatives or fragments thereof according to the present invention. The present invention includes, for example, monoclonal and polyclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, or the product of a Fab expression library. It is within the present invention that the antibody may be chimeric, i. e. that different parts thereof stem from different species or at least the respective sequences are taken from different species.

Antibodies generated against the hyperimmune serum reactive antigens and fragments thereof corresponding to a sequence of the present invention can be obtained by direct injection of the hyperimmune serum reactive antigens and fragments thereof into an animal or by administering the hyperimmune serum reactive antigens and fragments thereof to an animal, preferably a non-human. The antibody so obtained will then bind the hyperimmune serum reactive antigens and fragments thereof itself. In this manner, even a sequence encoding only a fragment of a hyperimmune serum reactive antigen and fragments thereof can be used to generate antibodies binding the whole native hyperimmune serum reactive antigen and fragments thereof. Such antibodies can then be used to isolate the hyperimmune serum reactive antigens and fragments thereof from tissue expressing those hyperimmune serum reactive antigens and fragments thereof.

For preparation of monoclonal antibodies, any technique known in the art, which provides antibodies produced by continuous cell line cultures can be used (as described originally in {Kohler, G. et al., 1975}.

Techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778) can be adapted to produce single chain antibodies to immunogenic hyperimmune serum reactive antigens and fragments thereof according to this invention. Also, transgenic mice, or other organisms such as other mammals, may be used to express humanized antibodies to immunogenic hyperimmune serum reactive antigens and fragments thereof according to this invention.

Alternatively, phage display technology or ribosomal display could be utilized to select antibody genes with binding activities towards the hyperimmune serum reactive antigens and fragments thereof either

from repertoires of PCR amplified v-genes of lymphocytes from humans screened for possessing respective target antigens or from naïve libraries {McCafferty, J. et al., 1990}; {Marks, J. et al., 1992}. The affinity of these antibodies can also be improved by chain shuffling {Clackson, T. et al., 1991}.

If two antigen binding domains are present, each domain may be directed against a different epitope – termed 'bispecific' antibodies.

The above-described antibodies may be employed to isolate or to identify clones expressing the hyperimmune serum reactive antigens and fragments thereof or purify the hyperimmune serum reactive antigens and fragments thereof of the present invention by attachment of the antibody to a solid support for isolation and/or purification by affinity chromatography.

Thus, among others, antibodies against the hyperimmune serum reactive antigens and fragments thereof of the present invention may be employed to inhibit and/or treat infections, particularly bacterial infections and especially infections arising from *S. agalactiae.*

Hyperimmune serum reactive antigens and fragments thereof include antigenically, epitopically or immunologically equivalent derivatives, which form a particular aspect of this invention. The term "antigenically equivalent derivative" as used herein encompasses a hyperimmune serum reactive antigen and fragments thereof or its equivalent which will be specifically recognized by certain antibodies which, when raised to the protein or hyperimmune serum reactive antigen and fragments thereof according to the present invention, interfere with the interaction between pathogen and mammalian host. The term "immunologically equivalent derivative" as used herein encompasses a peptide or its equivalent which when used in a suitable formulation to raise antibodies in a vertebrate, the antibodies act to interfere with the interaction between pathogen and mammalian host.

The hyperimmune serum reactive antigens and fragments thereof, such as an antigenically or immunologically equivalent derivative or a fusion protein thereof can be used as an antigen to immunize a mouse or other animal such as a rat or chicken. The fusion protein may provide stability to the hyperimmune serum reactive antigens and fragments thereof. The antigen may be associated, for example by conjugation, with an immunogenic carrier protein, for example bovine serum albumin (BSA) or keyhole limpet haemocyanin (KLH). Alternatively, an antigenic peptide comprising multiple copies of the protein or hyperimmune serum reactive antigen and fragments thereof, or an antigenically or immunologically equivalent hyperimmune serum reactive antigen and fragments thereof, may be sufficiently antigenic to improve immunogenicity so as to obviate the use of a carrier.

Preferably the antibody or derivative thereof is modified to make it less immunogenic in the individual. For example, if the individual is human the antibody may most preferably be "humanized", wherein the complimentarity determining region(s) of the hybridoma-derived antibody has been transplanted into a human monoclonal antibody, for example as described in {Jones, P. et al., 1986} or {Tempest, P. et al., 1991}.

The use of a polynucleotide of the invention in genetic immunization will preferably employ a suitable delivery method such as direct injection of plasmid DNA into muscle, delivery of DNA complexed with specific protein carriers, coprecipitation of DNA with calcium phosphate, encapsulation of DNA in various forms of liposomes, particle bombardment {Tang, D. et al., 1992}, {Eisenbraun, M. et al., 1993} and *in vivo* infection using cloned retroviral vectors {Seeger, C. et al., 1984}.

In a further aspect the present invention relates to a peptide binding to any of the hyperimmune serum reactive antigens and fragments thereof according to the present invention, and a method for the manufacture of such peptides whereby the method is characterized by the use of the hyperimmune serum reactive antigens and fragments thereof according to the present invention and the basic steps are

known to the one skilled in the art.

Such peptides may be generated by using methods according to the state of the art such as phage display or ribosome display. In case of phage display, basically a library of peptides is generated, in form of phages, and this kind of library is contacted with the target molecule, in the present case a hyperimmune serum reactive antigen and fragments thereof according to the present invention. Those peptides binding to the target molecule are subsequently removed, preferably as a complex with the target molecule, from the respective reaction. It is known to the one skilled in the art that the binding characteristics, at least to a certain extent, depend on the particularly realized experimental set-up such as the salt concentration and the like. After separating those peptides binding to the target molecule with a higher affinity or a bigger force, from the non-binding members of the library, and optionally also after removal of the target molecule from the complex of target molecule and peptide, the respective peptide(s) may subsequently be characterised. Prior to the characterisation optionally an amplification step is realized such as, e. g. by propagating the peptide encoding phages. The characterisation preferably comprises the sequencing of the target binding peptides. Basically, the peptides are not limited in their lengths, however, preferably peptides having a lengths from about 8 to 20 amino acids are preferably obtained in the respective methods. The size of the libraries may be about 10^2 to 10^{18}, preferably 10^8 to 10^{15} different peptides, however, is not limited thereto.

A particular form of target binding hyperimmune serum reactive antigens and fragments thereof are the so-called "anticalines" which are, among others, described in German patent application DE 197 42 706.

In a further aspect the present invention relates to functional nucleic acids interacting with any of the hyperimmune serum reactive antigens and fragments thereof according to the present invention, and a method for the manufacture of such functional nucleic acids whereby the method is characterized by the use of the hyperimmune serum reactive antigens and fragments thereof according to the present invention and the basic steps are known to the one skilled in the art. The functional nucleic acids are preferably aptamers and spiegelmers.

Aptamers are D-nucleic acids, which are either single stranded or double stranded and which specifically interact with a target molecule. The manufacture or selection of aptamers is, e.g. described in European patent EP 0 533 838. Basically the following steps are realized. First, a mixture of nucleic acids, i. e. potential aptamers, is provided whereby each nucleic acid typically comprises a segment of several, preferably at least eight subsequent randomised nucleotides. This mixture is subsequently contacted with the target molecule whereby the nucleic acid(s) bind to the target molecule, such as based on an increased affinity towards the target or with a bigger force thereto, compared to the candidate mixture. The binding nucleic acid(s) are/is subsequently separated from the remainder of the mixture. Optionally, the thus obtained nucleic acid(s) is amplified using, e.g. polymerase chain reaction. These steps may be repeated several times giving at the end a mixture having an increased ratio of nucleic acids specifically binding to the target from which the final binding nucleic acid is then optionally selected. These specifically binding nucleic acid(s) are referred to as aptamers. It is obvious that at any stage of the method for the generation or identification of the aptamers samples of the mixture of individual nucleic acids may be taken to determine the sequence thereof using standard techniques. It is within the present invention that the aptamers may be stabilized such as, e. g., by introducing defined chemical groups which are known to the one skilled in the art of generating aptamers. Such modification may for example reside in the introduction of an amino group at the 2'-position of the sugar moiety of the nucleotides. Aptamers are currently used as therapeutical agents. However, it is also within the present invention that the thus selected or generated aptamers may be used for target validation and/or as lead substance for the development of medicaments, preferably of medicaments based on small molecules. This is actually done by a competition assay whereby the specific interaction between the target molecule and the aptamer is inhibited by a candidate drug whereby upon replacement of the aptamer from the complex of target and aptamer it may be assumed that the respective drug candidate allows a specific inhibition of the

interaction between target and aptamer, and if the interaction is specific, said candidate drug will, at least in principle, be suitable to block the target and thus decrease its biological availability or activity in a respective system comprising such target. The thus obtained small molecule may then be subject to further derivatisation and modification to optimise its physical, chemical, biological and/or medical characteristics such as toxicity, specificity, biodegradability and bioavailability.

Spiegelmers and their generation or manufacture is based on a similar principle. The manufacture of spiegelmers is described in international patent application WO 98/08856. Spiegelmers are L-nucleic acids, which means that they are composed of L-nucleotides rather than D-nucleotides as aptamers are. Spiegelmers are characterized by the fact that they have a very high stability in biological systems and, comparable to aptamers, specifically interact with the target molecule against which they are directed. In the process of generating spiegelmers, a heterogeonous population of D-nucleic acids is created and this population is contacted with the optical antipode of the target molecule, in the present case for example with the D-enantiomer of the naturally occurring L-enantiomer of the hyperimmune serum reactive antigens and fragments thereof according to the present invention. Subsequently, those D-nucleic acids are separated which do not interact with the optical antipode of the target molecule. But those D-nucleic acids interacting with the optical antipode of the target molecule are separated, optionally identified and/or sequenced and subsequently the corresponding L-nucleic acids are synthesized based on the nucleic acid sequence information obtained from the D-nucleic acids. These L-nucleic acids which are identical in terms of sequence with the aforementioned D-nucleic acids interacting with the optical antipode of the target molecule, will specifically interact with the naturally occurring target molecule rather than with the optical antipode thereof. Similar to the method for the generation of aptamers it is also possible to repeat the various steps several times and thus to enrich those nucleic acids specifically interacting with the optical antipode of the target molecule.

In a further aspect the present invention relates to functional nucleic acids interacting with any of the nucleic acid molecules according to the present invention, and a method for the manufacture of such functional nucleic acids whereby the method is characterized by the use of the nucleic acid molecules and their respective sequences according to the present invention and the basic steps are known to the one skilled in the art. The functional nucleic acids are preferably ribozymes, antisense oligonucleotides and siRNA.

Ribozymes are catalytically active nucleic acids, which preferably consist of RNA, which basically comprises two moieties. The first moiety shows a catalytic activity whereas the second moiety is responsible for the specific interaction with the target nucleic acid, in the present case the nucleic acid coding for the hyperimmune serum reactive antigens and fragments thereof according to the present invention. Upon interaction between the target nucleic acid and the second moiety of the ribozyme, typically by hybridisation and Watson-Crick base pairing of essentially complementary stretches of bases on the two hybridising strands, the catalytically active moiety may become active which means that it catalyses, either intramolecularly or intermolecularly, the target nucleic acid in case the catalytic activity of the ribozyme is a phosphodiesterase activity. Subsequently, there may be a further degradation of the target nucleic acid, which in the end results in the degradation of the target nucleic acid as well as the protein derived from the said target nucleic acid. Ribozymes, their use and design principles are known to the one skilled in the art, and, for example described in {Doherty, E. et al., 2001} and {Lewin, A. et al., 2001}.

The activity and design of antisense oligonucleotides for the manufacture of a medicament and as a diagnostic agent, respectively, is based on a similar mode of action. Basically, antisense oligonucleotides hybridise based on base complementarity, with a target RNA, preferably with a mRNA, thereby activating RNase H. RNase H is activated by both phosphodiester and phosphorothioate-coupled DNA. Phosphodiester-coupled DNA, however, is rapidly degraded by cellular nucleases with the exception of phosphorothioate-coupled DNA. These resistant, non-naturally occurring DNA derivatives do not inhibit

RNase H upon hybridisation with RNA. In other words, antisense polynucleotides are only effective as DNA RNA hybride complexes. Examples for this kind of antisense oligonucleotides are described, among others, in US-patent US 5,849,902 and US 5,989,912. In other words, based on the nucleic acid sequence of the target molecule which in the present case are the nucleic acid molecules for the hyperimmune serum reactive antigens and fragments thereof according to the present invention, either from the target protein from which a respective nucleic acid sequence may in principle be deduced, or by knowing the nucleic acid sequence as such, particularly the mRNA, suitable antisense oligonucleotides may be designed base on the principle of base complementarity.

Particularly preferred are antisense-oligonucleotides, which have a short stretch of phosphorothioate DNA (3 to 9 bases). A minimum of 3 DNA bases is required for activation of bacterial RNase H and a minimum of 5 bases is required for mammalian RNase H activation. In these chimeric oligonucleotides there is a central region that forms a substrate for RNase H that is flanked by hybridising "arms" comprised of modified nucleotides that do not form substrates for RNase H. The hybridising arms of the chimeric oligonucleotides may be modified such as by 2′-O-methyl or 2′-fluoro. Alternative approaches used methylphosphonate or phosphoramidate linkages in said arms. Further embodiments of the antisense oligonucleotide useful in the practice of the present invention are P-methoxyoligonucleotides, partial P-methoxyoligodeoxyribonucleotides or P-methoxyoligonucleotides.

Of particular relevance and usefulness for the present invention are those antisense oligonucleotides as more particularly described in the above two mentioned US patents. These oligonucleotides contain no naturally occurring 5′→3′-linked nucleotides. Rather the oligonucleotides have two types of nucleotides: 2′-deoxyphosphorothioate, which activate RNase H, and 2′-modified nucleotides, which do not. The linkages between the 2′-modified nucleotides can be phosphodiesters, phosphorothioate or P-ethoxyphosphodiester. Activation of RNase H is accomplished by a contiguous RNase H-activating region, which contains between 3 and 5 2′-deoxyphosphorothioate nucleotides to activate bacterial RNase H and between 5 and 10 2′- deoxyphosphorothioate nucleotides to activate eucaryotic and, particularly, mammalian RNase H. Protection from degradation is accomplished by making the 5′ and 3′ terminal bases highly nuclease resistant and, optionally, by placing a 3′ terminal blocking group.

More particularly, the antisense oligonucleotide comprises a 5′ terminus and a 3′ terminus; and from position 11 to 59 5′→3′-linked nucleotides independently selected from the group consisting of 2′-modified phosphodiester nucleotides and 2′-modified P-alkyloxyphosphotriester nucleotides; and wherein the 5′-terminal nucleoside is attached to an RNase H-activating region of between three and ten contiguous phosphorothioate-linked deoxyribonucleotides, and wherein the 3′-terminus of said oligonucleotide is selected from the group consisting of an inverted deoxyribonucleotide, a contiguous stretch of one to three phosphorothioate 2′-modified ribonucleotides, a biotin group and a P-alkyloxyphosphotriester nucleotide.

Also an antisense oligonucleotide may be used wherein not the 5′ terminal nucleoside is attached to an RNase H-activating region but the 3′ terminal nucleoside as specified above. Also, the 5′ terminus is selected from the particular group rather than the 3′ terminus of said oligonucleotide.

The nucleic acids as well as the hyperimmune serum reactive antigens and fragments thereof according to the present invention may be used as or for the manufacture of pharmaceutical compositions, especially vaccines. Preferably such pharmaceutical composition, preferably vaccine is for the prevention or treatment of diseases caused by, related to or associated with S. *agalactiae*. In so far another aspect of the invention relates to a method for inducing an immunological response in an individual, particularly a mammal, which comprises inoculating the individual with the hyperimmune serum reactive antigens and fragments thereof of the invention, or a fragment or variant thereof, adequate to produce antibodies to protect said individual from infection, particularly *streptococcal* infection and most particularly *S. agalactiae* infections.

Yet another aspect of the invention relates to a method of inducing an immunological response in an individual which comprises, through gene therapy or otherwise, delivering a nucleic acid functionally encoding hyperimmune serum reactive antigens and fragments thereof, or a fragment or a variant thereof, for expressing the hyperimmune serum reactive antigens and fragments thereof, or a fragment or a variant thereof *in vivo* in order to induce an immunological response to produce antibodies or a cell mediated T cell response, either cytokine-producing T cells or cytotoxic T cells, to protect said individual from disease, whether that disease is already established within the individual or not. One-way of administering the gene is by accelerating it into the desired cells as a coating on particles or otherwise.

A further aspect of the invention relates to an immunological composition which, when introduced into a host capable of having induced within it an immunological response, induces an immunological response in such host, wherein the composition comprises recombinant DNA which codes for and expresses an antigen of the hyperimmune serum reactive antigens and fragments thereof of the present invention. The immunological response may be used therapeutically or prophylactically and may take the form of antibody immunity or cellular immunity such as that arising from CTL or CD4+ T cells.

The hyperimmune serum reactive antigens and fragments thereof of the invention or a fragment thereof may be fused with a co-protein which may not by itself produce antibodies, but is capable of stabilizing the first protein and producing a fused protein which will have immunogenic and protective properties. This fused recombinant protein preferably further comprises an antigenic co-protein, such as Glutathione-S-transferase (GST) or beta-galactosidase, relatively large co-proteins which solubilise the protein and facilitate production and purification thereof. Moreover, the co-protein may act as an adjuvant in the sense of providing a generalized stimulation of the immune system. The co-protein may be attached to either the amino or carboxy terminus of the first protein.

Also, provided by this invention are methods using the described nucleic acid molecule or particular fragments thereof in such genetic immunization experiments in animal models of infection with *S. agalactiae*. Such fragments will be particularly useful for identifying protein epitopes able to provoke a prophylactic or therapeutic immune response. This approach can allow for the subsequent preparation of monoclonal antibodies of particular value from the requisite organ of the animal successfully resisting or clearing infection for the development of prophylactic agents or therapeutic treatments of *S. agalactiae* infection in mammals, particularly humans.

The hyperimmune serum reactive antigens and fragments thereof may be used as an antigen for vaccination of a host to produce specific antibodies which protect against invasion of bacteria, for example by blocking adherence of bacteria to damaged tissue. Examples of tissue damage include wounds in skin or connective tissue and mucosal tissues caused e.g. by viral infection (esp. respiratory, such as the flu) mechanical, chemical or thermal damage or by implantation of indwelling devices, or wounds in the mucous membranes, such as the mouth, mammary glands, urethra or vagina.

The present invention also includes a vaccine formulation, which comprises the immunogenic recombinant protein together with a suitable carrier. Since the protein may be broken down in the stomach, it is preferably administered parenterally, including, for example, administration that is subcutaneous, intramuscular, intravenous, intradermal intranasal or tramsdermal. Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the bodily fluid, preferably the blood, of the individual; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials, and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the

formulation, such as oil-in-water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

According to another aspect, the present invention relates to a pharmaceutical composition comprising such a hyperimmune serum-reactive antigen or a fragment thereof as provided in the present invention for *S. agalactiae.* Such a pharmaceutical composition may comprise one preferably at least two or more hyperimmune serum reactive antigens or fragments thereof against *S. agalactiae.* Optionally, such *S. agalactiae* hyperimmune serum reactive antigens or fragments thereof may also be combined with antigens against other pathogens in a combination pharmaceutical composition. Preferably, said pharmaceutical composition is a vaccine for preventing or treating an infection caused by *S. agalactiae* and/or other pathogens against which the antigens have been included in the vaccine.

According to a further aspect, the present invention relates to a pharmaceutical composition comprising a nucleic acid molecule encoding a hyperimmune serum-reactive antigen or a fragment thereof as identified above for *S. agalactiae.* Such a pharmaceutical composition may comprise one or more nucleic acid molecules encoding hyperimmune serum reactive antigens or fragments thereof against *S. agalactiae.* Optionally, such *S. agalactiae* nucleic acid molecules encoding hyperimmune serum reactive antigens or fragments thereof may also be combined with nucleic acid molecules encoding antigens against other pathogens in a combination pharmaceutical composition. Preferably, said pharmaceutical composition is a vaccine for preventing or treating an infection caused by *S. agalactiae* and/or other pathogens against which the antigens have been included in the vaccine.

The pharmaceutical composition may contain any suitable auxiliary substances, such as buffer substances, stabilisers or further active ingredients, especially ingredients known in connection of pharmaceutical composition and/or vaccine production.

A preferable carrier/or excipient for the hyperimmune serum-reactive antigens, fragments thereof or a coding nucleic acid molecule thereof according to the present invention is an immunostimulatory compound for further stimulating the immune response to the given hyperimmune serum-reactive antigen, fragment thereof or a coding nucleic acid molecule thereof. Preferably the immunostimulatory compound in the pharmaceutical preparation according to the present invention is selected from the group of polycationic substances, especially polycationic peptides, immunostimulatory nucleic acids molecules, preferably immunostimulatory deoxynucleotides, alum, Freund's complete adjuvants, Freund's incomplete adjuvants, neuroactive compounds, especially human growth hormone, or combinations thereof.

It is also within the scope of the present invention that the pharmaceutical composition, especially vaccine, comprises apart from the hyperimmune serum reactive antigens, fragments thereof and/or coding nucleic acid molecules thereof according to the present invention other compounds which are biologically or pharmaceutically active. Preferably, the vaccine composition comprises at least one polycationic peptide. The polycationic compound(s) to be used according to the present invention may be any polycationic compound, which shows the characteristic effects according to the WO 97/30721. Preferred polycationic compounds are selected from basic polyppetides, organic polycations, basic polyamino acids or mixtures thereof. These polyamino acids should have a chain length of at least 4 amino acid residues (WO 97/30721). Especially preferred are substances like polylysine, polyarginine and polypeptides containing more than 20 %, especially more than 50 % of basic amino acids in a range of more than 8, especially more than 20, amino acid residues or mixtures thereof. Other preferred polycations and their pharmaceutical compositions are described in WO 97/30721 (e.g. polyethyleneimine) and WO 99/38528. Preferably these polypeptides contain between 20 and 500 amino acid residues, especially between 30 and 200 residues.

These polycationic compounds may be produced chemically or recombinantly or may be derived from

natural sources.

Cationic (poly)peptides may also be anti-microbial with properties as reviewed in {Ganz, T., 1999}. These (poly)peptides may be of prokaryotic or animal or plant origin or may be produced chemically or recombinantly (WO 02/13857). Peptides may also belong to the class of defensins (WO 02/13857). Sequences of such peptides can be, for example, found in the Antimicrobial Sequences Database under the following internet address:

http://www.bbcm.univ.trieste.it/~tossi/pag2.html

Such host defence peptides or defensives are also a preferred form of the polycationic polymer according to the present invention. Generally, a compound allowing as an end product activation (or down-regulation) of the adaptive immune system, preferably mediated by APCs (including dendritic cells) is used as polycationic polymer.

Especially preferred for use as polycationic substances in the present invention are cathelicidin derived antimicrobial peptides or derivatives thereof (International patent application WO 02/13857, incorporated herein by reference), especially antimicrobial peptides derived from mammalian cathelicidin, preferably from human, bovine or mouse.

Polycationic compounds derived from natural sources include HIV-REV or HIV-TAT (derived cationic peptides, antennapedia peptides, chitosan or other derivatives of chitin) or other peptides derived from these peptides or proteins by biochemical or recombinant production. Other preferred polycationic compounds are cathelin or related or derived substances from cathelin. For example, mouse cathelin is a peptide which has the amino acid sequence NH_2-RLAGLLRKGGEKIGEKLKKIGQKIKNFFQKLVPQPE-COOH. Related or derived cathelin substances contain the whole or parts of the cathelin sequence with at least 15-20 amino acid residues. Derivations may include the substitution or modification of the natural amino acids by amino acids which are not among the 20 standard amino acids. Moreover, further cationic residues may be introduced into such cathelin molecules. These cathelin molecules are preferred to be combined with the antigen. These cathelin molecules surprisingly have turned out to be also effective as an adjuvant for an antigen without the addition of further adjuvants. It is therefore possible to use such cathelin molecules as efficient adjuvants in vaccine formulations with or without further immunactivating substances.

Another preferred polycationic substance to be used according to the present invention is a synthetic peptide containing at least 2 KLK-motifs separated by a linker of 3 to 7 hydrophobic amino acids (International patent application WO 02/32451, incorporated herein by reference).

The pharmaceutical composition of the present invention may further comprise immunostimulatory nucleic acid(s). Immunostimulatory nucleic acids are e. g. neutral or artificial CpG containing nucleic acids, short stretches of nucleic acids derived from non-vertebrates or in form of short oligonucleotides (ODNs) containing non-methylated cytosine-guanine di-nucleotides (CpG) in a certain base context (e.g. described in WO 96/02555). Alternatively, also nucleic acids based on inosine and cytidine as e.g. described in the WO 01/93903, or deoxynucleic acids containing deoxy-inosine and/or deoxyuridine residues (described in WO 01/93905 and PCT/EP 02/05448, incorporated herein by reference) may preferably be used as immunostimulatory nucleic acids for the present invention. Preferablly, the mixtures of different immunostimulatory nucleic acids may be used according to the present invention.

It is also within the present invention that any of the aforementioned polycationic compounds is combined with any of the immunostimulatory nucleic acids as aforementioned. Preferably, such combinations are according to the ones as described in WO 01/93905, WO 02/32451, WO 01/54720, WO

01/93903, WO 02/13857 and PCT/EP 02/05448 and the Austrian patent application A 1924/2001, incorporated herein by reference.

In addition or alternatively such vaccine composition may comprise apart from the hyperimmune serum reactive antigens and fragments thereof, and the coding nucleic acid molecules thereof according to the present invention a neuroactive compound. Preferably, the neuroactive compound is human growth factor as, e.g. described in WO 01/24822. Also preferably, the neuroactive compound is combined with any of the polycationic compounds and/or immunostimulatory nucleic acids as afore-mentioned.

In a further aspect the present invention is related to a pharmaceutical composition. Such pharmaceutical composition is, for example, the vaccine described herein. Also a pharmaceutical composition is a pharmaceutical composition which comprises any of the following compounds or combinations thereof: the nucleic acid molecules according to the present invention, the hyperimmune serum reactive antigens and fragments thereof according to the present invention, the vector according to the present invention, the cells according to the present invention, the antibody according to the present invention, the functional nucleic acids according to the present invention and the binding peptides such as the anticalines according to the present invention, any agonists and antagonists screened as described herein. In connection therewith any of these compounds may be employed in combination with a non-sterile or sterile carrier or carriers for use with cells, tissues or organisms, such as a pharmaceutical carrier suitable for administration to a subject. Such compositions comprise, for instance, a media additive or a therapeutically effective amount of a hyperimmune serum reactive antigen and fragments thereof of the invention and a pharmaceutically acceptable carrier or excipient. Such carriers may include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol and combinations thereof. The formulation should suit the mode of administration.

The pharmaceutical compositions may be administered in any effective, convenient manner including, for instance, administration by topical, oral, anal, vaginal, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal, intratracheal or intradermal routes among others.

In therapy or as a prophylactic, the active agent may be administered to an individual as an injectable composition, for example as a sterile aqueous dispersion, preferably isotonic.

Alternatively the composition may be formulated for topical application, for example in the form of ointments, creams, lotions, eye ointments, eye drops, ear drops, mouthwash, impregnated dressings and sutures and aerosols, and may contain appropriate conventional additives, including, for example, preservatives, solvents to assist drug penetration, and emollients in ointments and creams. Such topical formulations may also contain compatible conventional carriers, for example cream or ointment bases, and ethanol or oleyl alcohol for lotions. Such carriers may constitute from about 1 % to about 98 % by weight of the formulation; more usually they will constitute up to about 80 % by weight of the formulation.

In addition to the therapy described above, the compositions of this invention may be used generally as a wound treatment agent to prevent adhesion of bacteria to matrix proteins exposed in wound tissue and for prophylactic use in dental treatment as an alternative to, or in conjunction with, antibiotic prophylaxis.

A vaccine composition is conveniently in injectable form. Conventional adjuvants may be employed to enhance the immune response. A suitable unit dose for vaccination is 0.05-5 μg antigen / per kg of body weight, and such dose is preferably administered 1-3 times and with an interval of 1-3 weeks.

With the indicated dose range, no adverse toxicological effects should be observed with the compounds of the invention, which would preclude their administration to suitable individuals.

In a further embodiment the present invention relates to diagnostic and pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention. The ingredient(s) can be present in a useful amount, dosage, formulation or combination. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, reflecting approval by the agency of the manufacture, use or sale of the product for human administration.

In connection with the present invention any disease related use as disclosed herein such as, e. g. use of the pharmaceutical composition or vaccine, is particularly a disease or diseased condition which is caused by, linked or associated with Streptococci, more preferably, *S. pyogens and pneumoniae*. In connection therewith it is to be noted that *S. agalactiae* comprises several strains including those disclosed herein. A disease related, caused or associated with the bacterial infection to be prevented and/or treated according to the present invention includes besides others bacterial pharyngitis, otitis media, pneumonia, bacteremia, meningitis, peritonitis, wound infection and sepsis in humans.

In a still further embodiment the present invention is related to a screening method using any of the hyperimmune serum reactive antigens or nucleic acids according to the present invention. Screening methods as such are known to the one skilled in the art and can be designed such that an agonist or an antagonist is screened. Preferably an antagonist is screened which in the present case inhibits or prevents the binding of any hyperimmune serum reactive antigen and fragment thereof according to the present invention to an interaction partner. Such interaction partner can be a naturally occurring interaction partner or a non-naturally occurring interaction partner.

The invention also provides a method of screening compounds to identify those, which enhance (agonist) or block (antagonist) the function of hyperimmune serum reactive antigens and fragments thereof or nucleic acid molecules of the present invention, such as its interaction with a binding molecule. The method of screening may involve high-throughput.

For example, to screen for agonists or antagonists, the interaction partner of the nucleic acid molecule and nucleic acid, respectively, according to the present invention, maybe a synthetic reaction mix, a cellular compartment, such as a membrane, cell envelope or cell wall, or a preparation of any thereof, may be prepared from a cell that expresses a molecule that binds to the hyperimmune serum reactive antigens and fragments thereof of the present invention. The preparation is incubated with labelled hyperimmune serum reactive antigens and fragments thereof in the absence or the presence of a candidate molecule, which may be an agonist or antagonist. The ability of the candidate molecule to bind the binding molecule is reflected in decreased binding of the labelled ligand. Molecules which bind gratuitously, i. e., without inducing the functional effects of the hyperimmune serum reactive antigens and fragments thereof, are most likely to be good antagonists. Molecules that bind well and elicit functional effects that are the same as or closely related to the hyperimmune serum reactive antigens and fragments thereof are good agonists.

The functional effects of potential agonists and antagonists may be measured, for instance, by determining the activity of a reporter system following interaction of the candidate molecule with a cell or appropriate cell preparation, and comparing the effect with that of the hyperimmune serum reactive antigens and fragments thereof of the present invention or molecules that elicit the same effects as the hyperimmune serum reactive antigens and fragments thereof. Reporter systems that may be useful in this regard include but are not limited to colorimetric labelled substrate converted into product, a reporter gene that is responsive to changes in the functional activity of the hyperimmune serum reactive antigens and fragments thereof, and binding assays known in the art.

Another example of an assay for antagonists is a competitive assay that combines the hyperimmune serum reactive antigens and fragments thereof of the present invention and a potential antagonist with membrane-bound binding molecules, recombinant binding molecules, natural substrates or ligands, or substrate or ligand mimetics, under appropriate conditions for a competitive inhibition assay. The hyperimmune serum reactive antigens and fragments thereof can be labelled such as by radioactivity or a colorimetric compound, such that the molecule number of hyperimmune serum reactive antigens and fragments thereof bound to a binding molecule or converted to product can be determined accurately to assess the effectiveness of the potential antagonist.

Potential antagonists include small organic molecules, peptides, polypeptides and antibodies that bind to a hyperimmune serum reactive antigen and fragments thereof of the invention and thereby inhibit or extinguish its acitivity. Potential antagonists also may be small organic molecules, a peptide, a polypeptide such as a closely related protein or antibody that binds to the same sites on a binding molecule without inducing functional activity of the hyperimmune serum reactive antigens and fragments thereof of the invention.

Potential antagonists include a small molecule, which binds to and occupies the binding site of the hyperimmune serum reactive antigens and fragments thereof thereby preventing binding to cellular binding molecules, such that normal biological activity is prevented. Examples of small molecules include but are not limited to small organic molecules, peptides or peptide-like molecules.

Other potential antagonists include antisense molecules (see {Okano, H. et al., 1991}; OLIGODEOXYNUCLEOTIDES AS ANTISENSE INHIBITORS OF GENE EXPRESSION; CRC Press, Boca Ration, FL (1988), for a description of these molecules).

Preferred potential antagonists include derivatives of the hyperimmune serum reactive antigens and fragments thereof of the invention.

As used herein the activity of a hyperimmune serum reactive antigen and fragment thereof according to the present invention is its capability to bind to any of its interaction partner or the extent of such capability to bind to its or any interaction partner.

In a particular aspect, the invention provides the use of the hyperimmune serum reactive antigens and fragments thereof, nucleic acid molecules or inhibitors of the invention to interfere with the initial physical interaction between a pathogen and mammalian host responsible for sequelae of infection. In particular the molecules of the invention may be used: i) in the prevention of adhesion of *S. agalactiae* to mammalian extracellular matrix proteins at mucosal surfaces and on in-dwelling devices or to extracellular matrix proteins in wounds; ii) to block bacterial adhesion between mammalian extracellular matrix proteins and bacterial proteins which mediate tissue damage or invasion iii) or lead to evasion of immune defense; iv) to block the normal progression of pathogenesis in infections initiated other than by the implantation of in-dwelling devices or by other surgical techniques, e.g. through inhibiting nutrient acquisition.

Each of the DNA coding sequences provided herein may be used in the discovery and development of antibacterial compounds. The encoded protein upon expression can be used as a target for the screening of antibacterial drugs. Additionally, the DNA sequences encoding the amino terminal regions of the encoded protein or Shine-Delgarno or other translation facilitating sequences of the respective mRNA can be used to construct antisense sequences to control the expression of the coding sequence of interest.

The antagonists and agonists may be employed, for instance, to inhibit diseases arising from infection with Streptococcus, especially *S. agalactiae*, such as sepsis.

In a still further aspect the present invention is related to an affinity device such affinity device comprises as least a support material and any of the hyperimmune serum reactive antigens and fragments thereof according to the present invention, which is attached to the support material. Because of the specificity of the hyperimmune serum reactive antigens and fragments thereof according to the present invention for their target cells or target molecules or their interaction partners, the hyperimmune serum reactive antigens and fragments thereof allow a selective removal of their interaction partner(s) from any kind of sample applied to the support material provided that the conditions for binding are met. The sample may be a biological or medical sample, including but not limited to, fermentation broth, cell debris, cell preparation, tissue preparation, organ preparation, blood, urine, lymph liquid, liquor and the like.

The hyperimmune serum reactive antigens and fragments thereof may be attached to the matrix in a covalent or non-covalent manner. Suitable support material is known to the one skilled in the art and can be selected from the group comprising cellulose, silicon, glass, aluminium, paramagnetic beads, starch and dextrane.

The present invention is further illustrated by the following figures, examples and the sequence listing, from which further features, embodiments and advantages may be taken. It is to be understood that the present examples are given by way of illustration only and not by way of limitation of the disclosure.

In connection with the present invention

Figure 1 shows the characterization of human antibody sources for *S. agalactiae*.

Figure 2 shows the characterization of the small fragment genomic library, LSAg-70, from *Streptococcus agalactiae* ATCC 12403.

Figure 3 shows the selection of bacterial cells by MACS using biotinylated human IgGs.

Figure 4 shows the serotypes of the applied strains and an example for the gene distribution analysis with one of the identified antigens.

Figure 5 shows examples for induction of epitope-specific antibodies in mice by immunization with *E. coli* lysates.

Figure 6 shows examples for cell surface staining with epitope-specific antisera by flow cytometry.

Figure 7 shows the determination of bactericidal activity of antibodies induced by selected epitopes in an *in vitro* assay.

Table 1A shows the summary of all screens performed with genomic *S. agalactiae* libraries and human serum. Table 1B shows antigenic proteins identified by sequence identity within antigenic regions of the proteins listed in Table 1A.

Table 2 shows the summary of epitope serology analysis with human sera.

Table 3 shows the summary of the gene distribution analysis for the identified antigens in 46 *S. agalactiae* strains.

Table 4 shows the summary of mouse imunogenicity experiments.

Table 5 shows the summary of all screens performed with genomic *S. agalactiae* libraries and human serum.

Table 6 shows the summary of epitope serology analysis with human sera.

Table 7 shows the summary of mouse imunogenicity experiments.

The figures to which it might be referred to in the specification are described in the following in more details.

Figure 1 shows the characterization of human sera and cervical secretions for anti-*S. agalactiae* antibodies as measured by immune assays. Total anti-*S. agalactiae* IgG and IgA antibody levels were measured by standard ELISA using total bacterial lysates or culture supernant fractions prepared from *S. agalactiae* serotype III strain ATCC 12403 as coating antigens. **(A)** Results of representative experiments are shown with healthy adult sera with total bacterial lysate proteins. Data are expressed as ELISA units calculated from absorbance at 405nm at a serum dilution in the linear range of detection (2.000X for IgA, 10,000 for IgG). Selected sera (out of 52) included in the healthy adult non-pregnant serum pool (NSag8-IgG,-IgA) are indicated by bold numbers. **(B)** Immunoblot analysis was performed on high titer sera selected by ELISA in order to ensure multiple immune reactivity with protein antigens. Results of a representative experiment using total bacterial lysate prepared from *S. agalactiae* serotype III ATCC 12403 strain and selected patients' sera at 5.000X dilution are shown. Blots were developed with anti-human IgG secondary antibody reagent. Low titer sera were included as negative controls. Mw: molecular weight markers. **(C)** shows selection of cervical secretions from noncolonized pregnant women by immunoblot analysis. Antibodies exctracted from cervical wicks were quantitated for IgA content. 2 μg IgA from each prepaprations were tested for immunoreactivity using total bacterial lysate in a multi-well blotting apparatus. Blots were developed with anti-human IgA secondary antibodies. IgA preparation showing reactivity with GBS proteins (inidicated by arrows) were selected and pooled.

Figure 2 (A) shows the fragment size distribution of the *Streptococcus agalactiae* ATCC 12403 small fragment genomic library, LSAg-70. After sequencing 576 randomly selected clones, sequences were trimmed (464) to eliminate vector residues and the numbers of clones with various genomic fragment sizes were plotted. **(B)** shows the graphic illustration of the distribution of the same set of randomly sequenced clones of LSAg-70 over the *S. agalactiae* ATCC 12403 chromosome. Rectangles indicate matching sequences to annotated ORFs and diamonds represent fully matched clones to non-coding chromosomal sequences in +/+ or +/- orientation. Circles position all clones with chimeric sequences. Numeric distances in base pairs are indicated over the circular genome for orientation. Partitioning of various clone sets within the library is given in numbers and percentage at the bottom of the figure.

Figure 3 (A) shows the MACS selection with biotinylated human IgGs. The LSAg-70 library in pMAL9.1 was screened with 15-20 μg biotinylated IgG (PSag11-IgG, purified from human serum). As negative control, no serum was added to the library cells for screening. Number of cells selected after the 1st, 2nd and 3rd elution are shown for each selection round (upper, middle and lower panel, respectively). **(B)** shows the reactivity of specific clones (1-26) selected by bacterial surface display as analysed by immunoblot analysis with the human serum IgG pool (PSag11-IgG, 4 μg/μl) used for selection by MACS at a dilution of 1:3,000. As a loading control the same blot was also analysed with antibodies directed against the platform protein LamB at a dilution of 1:5,000 of hyperimmune rabbit serum. M, Molecular weigth marker; L, Extract from a clone expressing LamB without foreign peptide insert.

Figure 4 (A) shows the representation of different serotypes of *S. agalactiae* clinical isolates analysed for the gene distribution study. A number of the strains were not typable and may represent additional serotypes. **(B)** shows the PCR analysis for the gene distribution of gbs0061 with the respective oligonucleotides and 46 *S. agalactiae* strains. The predicted size of the PCR fragments is 814 bp. 1-46, *S. agalactiae* strains, clinical isolates as shown under A; -, no genomic DNA added; +, genomic DNA from *S.*

agalactiae ATCC 12403, which served as template for library construction.

Figure 5 shows the measurement of epitope-specific mouse serum IgG antibody levels induced by total bacterial lysates of Lamb or FhuA expressing *E. coli* clones with *S. agalactiae*-derived epitopes. **(A)** shows representative peptide ELISA experiments with three sets of mouse sera (5 mice in each group, 1-5) generated by gbs0428, gbs0628 and gbs632 epitopes, respectively. Sera were tested at two different dilutions: black bars: 100X; grey bars; 1000X. Biotin-labeled synthetic peptides corresponding to the respective epitopes were used in the peptide ELISA. Sera induced with E. coli lysate without *S. agalactiae* derived epitopes are indicated as FhuA or LamB. **(B)** shows a typical immunoblotting experiment using lysates prepared from individual *E. coli* clones selected for mouse injections. Sera were depleted by *E. coli* lysate not carrying epitope to remove antibodies against *E. coli* proteins. Examples are shown for gbs0918, gbs0428, gbs0628 and gbs632 epitopes. Negative controls (-) are *E. coli* clones with empty platform proteins. Location of platform proteins LamB and FhuA is indicated by arrows.

Figure 6 shows the detection of specific antibody binding on the cell surface of *Streptococcus agalactiae* by flow cytometry. In Figure 6**A** preimmune mouse sera and polyclonal sera raised against *S. agalactiae* serotype III lysate were incubated with *S. agalactiae* strain serotype III and analysed by flow cytometry. Control shows the level of non-specific binding of the secondary antibody to the surface of *S. agalactiae* cells. The histograms in figure 6**B** indicates the increased fluorescence due to specific binding of anti-gbs0031, anti-gbs1925 and anti-gbs0012 antibodies in comparison to the control sera generated against E. coli lysate containing only the 'empty' platform protein FhuA.

Figure 7 shows the bactericidal activity of epitope specific antibodies as determined in *in vitro* killing assay. The killing activity of immune sera is measured parallel with and calculated relative to the appropriate control sera. Data are expressed as percentage of killing, that is the reduction on bacterial cfu numbers as a consequence of the presence of specific antibodies. Hyperimmune polyclonal mouse sera generated with *S. agalactiae* lysate and sera from non-immunized mice served as positive and negative controls for the assay, respectively. Immune sera generated with (A) gbs0012, gbs0016, gbs0031, gbs0428, gbs1306 and gbs2018 epitopes and with (B) gbs0233, gbs0419, gbs0942, gbs0975, gbs1038, gbs1144 and gbs2093 epitopes were tested for bactericidal activity and data are expressed relative to appropriate controls, such as sera induced with Lamb or FhuA expressing *E. coli* clones without *S. agalactiae*-derived epitopes. *S. agalactiae* serotype III cells were incubated with mouse phagocytic cells for 60 min, and surviving bacteria were quantified by counting cfus after plating on blood agar.

Table 1: Immunogenic proteins identified by bacterial surface display.
(A) Columns A, 300bp library of *S.agalactiae* ATCC 12403 in fhuA with NSag8-IgA (826), **B**, 300bp library in fhuA with PSag10-IgA (768), **C**, 300bp library in fhuA with PSag10-IgG (711), **D**, 300bp library in fhuA with PSag11-IgG (640), **E**, 70bp library in lamB with NSag8-IgA (1057), **F**, 70bp library in lamB with NSag8-IgG (869), **G**, 70bp library in lamB with PSag10-IgA (904), **H**, 70bp library in lamB with PSag10-IgA-adsorbed (493), **I**, 70bp library in lamB with PSag10-IgG (910), **J**, 70bp library in lamB with PSag11-IgA (631), **K**, 70bp library in lamB with PSag11-IgG (926), **L**, 70bp library in lamB with PSag18-IgA (691), **M**, 70bp library in lamB with PSag-sIgA (628); *, prediction of antigenic sequences longer than 5 amino acids was performed with the program ANTIGENIC (Kolaskar and Tongaonkar, 1990). **Table 1B** lists the immunogenic proteins identified by amino acid sequence identity with peptides identified by bacterial surface display. Antigenic peptides, which have been identified by bacterial surface-display possess identical counterparts in the listed proteins from *S. agalactiae*. The peptides have been shown to react with multiple human sera (see Table 2). Sera directed against these peptides can therefore recognize multiple proteins.

Table 2: Epitope serology with human sera.
Immune reactivity of individual synthetic peptides representing selected epitopes with human sera is shown. Extent of reactivity is expressed as +, ++ or +++, and summed from individual reactivities of

peptides with individual sera (13 patient and 9 healthy adult, 22 total). A total score for each peptide was calculated based on ELISA units as the sum of all reactivities. Scores were 2-8 for +, 9-16 for ++ and 17-26 for +++. ELISA units were calculated from OD_{405nm} readings and the serum dilution after correction for background. Location of synthetic peptides within the antigenic ORFs according to the genome annotation of ATCC 12403 strain is given in columns **aa from** and **aa to** indicating the first and last amino acid residues, respectively. Peptide names: gbs0012.1 present in annotated ORF: gbs0012.

Table 3: Gene distribution in *S. agalactiae* strains.
Fourty six *S. agalactiae* strains as shown in Figure 4A were tested by PCR with oligonucleotides specific for the genes encoding relevant antigens. The PCR fragment of one selected PCR reaction was sequenced in order to confirm the amplification of the correct DNA fragment. *, number of amino acid substitutions in a serotype IA strain as derived from sequencing as compared to *S. agalactiae* ATCC 12403. #, alternative strain used for sequencing, because gene was not present in the serotype IA strain.

Table 4: Immunogenicity of antigenic epitopes.
S. agalactiae antigens were tested for immunogenicity by immunization with *E. coli* clones harboring plasmids encoding the platform proteins LamB or FhuA fused to *S. agalactiae* peptides. The presence of epitope-specific antibodies were detected and measured by peptide ELISA and/or immunoblotting using the corresponding *E. coli* clone lysate, which served as immunogen. Results are expressed as + to +++++, and calculated for peptide ELISA as the sum of the reactivity of individual mouse sera based on ELISA units (as indicated on Fig. 5A) and for immunoblotting (**IB**) as the strength of reactivity of pooled (5 individual) mouse sera with the epitope containing platform protein (as indicated on Fig. 5B). Location of synthetic peptides within the antigenic ORFs according to the genome annotation of ATCC 12403 strain is given in columns **aa from** and **aa to** indicating the first and last amino acid residues, respectively.

Table 5: Immunogenic proteins identified by bacterial surface display.
(A) 300bp library of *S.agalactiae* ATCC 12403 in fhuA with IC8-IgA (826), B, 300bp library in fhuA with P10-IgA (768), C, 300bp library in fhuA with P10-IgG (711), D, 300bp library in fhuA with P11-IgG (640), E, 70bp library in lamB with IC8-IgA (1057), F, 70bp library in lamB with IC8-IgG (869), G, 70bp library in lamB with P10-IgA (904), H, 70bp library in lamB with P10-IgA-adsorbed (493), I, 70bp library in lamB with P10-IgG (910), J, 70bp library in lamB with P11-IgA (631), K, 70bp library in lamB with P11-IgG (926), *, prediction of antigenic sequences longer than 5 amino acids was performed with the program ANTIGENIC (Kolaskar and Tongaonkar, 1990).

Table 6: Epitope serology with human sera.
Immune reactivity of individual synthetic peptides representing selected epitopes with human sera is shown. Extent of reactivity is expressed as +, ++ or +++, and summed from individual reactivities of peptides with individual sera (13 patient and 9 healthy adult, 22 total). A total score for each peptide was calculated based on ELISA units as the sum of all reactivities. Scores were 2-8 for +, 9-16 for ++ and 17-30 for +++. ELISA units were calculated from OD_{405nm} readings and the serum dilution after correction for background. Location of synthetic peptides within the antigenic ORFs according to the genome annotation of ATCC 12403 strain is given in columns **aa from** and **aa to** indicating the first and last amino acid residues, respectively. Peptide names: gbs0233.1 present in annotated ORF: gbs0233.

Table 7: Immunogenicity of antigenic epitopes in mice.
S. agalactiae antigens were tested for immunogenicity by immunization with *E. coli* clones harboring plasmids encoding the platform proteins LamB or FhuA fused to *S. agalactiae* peptides. The presence of epitope-specific antibodies were detected and measured by peptide ELISA. Results are expressed as + to +++++, and calculated for peptide ELISA as the sum of the reactivity of individual mouse sera based on ELISA units (as indicated on Fig. 5). Location of epitopes within the antigenic ORFs according to the genome annotation of ATCC 12403 strain is given in columns **aa from** and **aa to** indicating the first and last amino acid residues, respectively.

EXAMPLES

Example 1: Characterization and selection of human serum sources based on anti-*S. agalactiae* antibodies, preparation of antibody screening reagents

Experimental procedures

Enzyme linked immune assay (ELISA).
ELISA plates (Maxisorb, Millipore) were coated with 5-10 μg/ml total protein diluted in coating buffer (0.1M sodium carbonate pH 9.2). Three dilutions of sera (2,000X, 10,000X, 50,000X) were made in PBS-BSA. Highly specific Horse Radish Peroxidase (HRP)-conjugated anti-human IgG or anti-human IgA secondary antibodies (Southern Biotech) were used according to the manufacturers' recommendations (dilution: 1,000x). Antigen-antibody complexes were quantified by measuring the conversion of the substrate (ABTS) to colored product based on OD_{405nm} readings by automatic ELIAS reader (TECAN SUNRISE).

Preparation of bacterial antigen extracts
Total bacterial lysate: Bacteria were grown overnight in THB (Todd-Hewitt Broth) and lysed by repeated freeze-thaw cycles: incubation on dry ice/ethanol-mixture until frozen (1 min), then thawed at 37°C (5 min): repeated 3 times. This was followed by sonication and collection of supernatant by centrifugation (3,500 rpm, 15 min, 4°C).
Culture supernatant: After removal of bacteria by centrifugation, the supernatant of overnight grown bacterial cultures was precipitated with ice-cold ethanol by mixing 1 part supernatant with 3 parts absolute ethanol and incubated overnight at -20°C. Precipitates were collected by centrifugation (2,600 g, for 15 min). Dry pellets were dissolved either in PBS for ELISA, or in urea and SDS-sample buffer for SDS-PAGE and immunoblotting. The protein concentration of samples was determined by Bradford assay.

Immunoblotting
Total bacterial lysate and culture supernatant samples were prepared from *in vitro* grown *S. agalactiae* serotype III strain. 10 to 25μg total protein/lane was separated by SDS-PAGE using the BioRad Mini-Protean Cell electrophoresis system and proteins transferred to nitrocellulose membrane (ECL, Amersham Pharmacia). After overnight blocking in 5% milk, human sera were added at 2,000x dilution, and HRPO labeled anti-human IgG was used for detection.

Extraction of antibodies from cervical wicks
Cervical secretions were collected by absorbent cylindrical wicks (Polyfiltronics) which were introduced into the cervical canal during speculum examination and thereafter kept frozen until extraction. Extraction was done according to Hordnes *et al*, 1998 (provider of the samples). Briefly, wicks were mixed with PBS containing protease inhibitors, vortexed and fluid was drained from the tubes containing the wicks. The concentrations of total IgA and IgG antibodies in extracts were determined.

Purification of antibodies for genomic screening. Five sera from both the patient and the healthy group were selected based on the overall anti-GBS titers for serum or cervical secretion pools used in the screening procedure. Antibodies against *E. coli* proteins were removed by incubating the heat-inactivated sera with whole cell *E. coli* cells (DH5alpha, transformed with pHIE11, grown under the same condition as used for bacterial surface display). Highly enriched preparations of IgGs from the pooled, depleted sera were generated by protein G affinity chromatography, according to the manufacturer's instructions (UltraLink Immobilized Protein G, Pierce). IgA antibodies were purified also by affinity chromatography using biotin-labeled anti-human IgA (Southern Biotech) immobilized on Streptavidin-agarose (GIBCO BRL). The efficiency of depletion and purification was checked by SDS-PAGE, Western blotting, ELISA and protein concentration measurements.

Results

The antibodies produced against *S. agalactiae* by the human immune system and present in human sera are indicative of the *in vivo* expression of the antigenic proteins and their immunogenicity. These molecules are essential for the identification of individual antigens in the approach as described in the present invention, which is based on the interaction of the specific anti-GBS antibodies and the corresponding *S. agalactiae* peptides or proteins. To gain access to relevant antibody repertoires, human sera were collected from

I. healthy pregnant women tested negative for cervical and anorectal carriage of GBS
II. healthy pregnant women tested positive for cervical and/or anorectal carriage of GBS who's newborn remained GBS-free (although with antibiotic prevention).
III. adults below <45 years of age without clinical disease.
IV. naïve individuals, youg children between 5 and 10 months of age, after they already lost maternal antibodies and have not acquired GBS-specific ones due to the lack of GBS disease.

In addition cervical secretions were also collected from the first two groups of donors. The extrem value of these antibody sources is mainly the secretory IgA component, which is directly implicated as protective effector moluecule on mucosal surfaces.

It is important to screen with antibodies from at least two different populations, pregnant women and nonpregnant adults, since GBS disease affects elderly and immunocompromised adults, as well. Within the pregnant study group, there are again two different patient categories, women who are GBS colonized and those who are noncolonized, to be included in the antigen screen.

Antibodies in serum and other body fluids, such as mucosal secretions induced in individuals exposed to the pathogens are crucial for antigen identification. The exposure to GBS results in asymptomatic colonization, current or past acute or chronic infection. *S. agalactiae* colonization and infections are common, and antibodies are present as a consequence of natural immunization from previous encounters. It is likely that sera from high titer noncolonized individuals contain functional antibodies, which are able to eliminate carriage. At the same time certain antibodies might be induced against GBS components only if the antigen persist. For that reason sera from colonized individuals were also included. It has been shown that colonization is associated with capsular polysaccahride (CPS)-specific antibody responses. However, it is not clear whether sufficient level of antibodies to CPS's would prevent GBS colonization, since there are colonized women with both high and low levels of anti-CPS antibody, and the same is true for noncolonized pregnant women.

However, there are reports that effector function and avidity of antibodies produced during pregnancy might be altered. It is important to recognize that most healthy adults are protected from invasive GBS disease and are less susceptible than newborns and the elderly. Antibodies from these individuals seem to be especially valuable for identification of the corresponding antigens. It is known that anti-GBS antibody levels increase with age.

GBS is a mucosal pathogen and should induce IgA response; for that reason it was important to perform IgA-based screens, as well as IgG-based screens. The fact that some *S. agalactiae* strains express high affinity IgA-binding receptor also points to the importance of IgA in host response. Recently it was reported that not only IgG, but also IgA serum antibodies can be recognized by the FcRIII receptors of PMNs and promote opsonization {Phillips-Quagliata, J. et al., 2000}; {Shibuya, A. et al., 2000}. The primary role of IgA antibodies is neutralization, mainly at the mucosal surface. The level of serum IgA reflects the quality, quantity and specificity of the dimeric secretory IgA. For that reason the serum collection was not only analyzed for anti-streptococcal IgG, but also for IgA levels. In the ELISA assays highly specific secondary reagents were used to detect antibodies from the high affinity types, such as

IgG and IgA, but avoided IgM. Production of IgM antibodies occurs during the primary adaptive humoral response, and results in low affinity antibodies, while IgG and IgA antibodies had already undergone affinity maturation, and are more valuable in fighting or preventing disease.

127 serum samples and 97 cervical secretions from pregnant women and 50 sera from healthy adults were characterized for anti-*S. agalactiae* antibodies by a series of immune assays. Primary characterization was done by ELISA using two different antigen preparations, such as total bacterial extract and culture supernatant proteins prepared from *S. agalactiae* serotype III ATCC 12403 strain. A representative experiment is shown in Fig. 1A using sera from the healthy adult population. Antibody titers were compared at given dilutions where the response was linear. Sera were ranked based on the IgG and IgA reactivity against the two complex antigenic mixtures (including serotype specific type III capsule), and the highest ones were selected for further testing by immunoblotting. This analysis confirmed a high antibody reactivity of the pre-selected sera against multiple GBS proteins, especially when compared to not selected, low-titer sera (Fig 1B). However, ELISA ranking of sera did not always correlated with immunoblot signals suggesting that anti-capsular antibodies were abundant and dominated the ELISA reactivities against total bacterial extracts. Thus the final selection of sera to be included in antibody-pools was based mainly on multiple immunogenic bands in immunoblotting experiments. This extensive antibody characterization approach has led to the unambiguous identification of anti-GBS hyperimmune sera.

The 97 cervical secretions were determined for IgA content, and same amount (2μg) was tested for anti-GBS reactivity by immunoblotting. Positively selected sera (as it is shown in Fig. 1C) were devided into colonized and noncolonized IgA pools and used separately in bacterial surface display experiments.

5 sera from both donor groups were selected and pooled for antigen identification by bacterial surface display. Selected sera included in the four pregnant women pools (PSAg10-IgG,-IgA, PSAg11-IgG, PSAg18-IgG and PSAg-sIgA) and one healthy adult (non-pregnant) pool (NSAg8-IgG,-IgA). IgG and IgA antibodies were purified from pooled sera by affinity chromatography and depleted of E. coli -reactive antibodies to avoid background in the bacterial surface display screen.

Example 2: Generation of highly random, frame-selected, small-fragment, genomic DNA libraries of *Streptococcus agalactiae*

Experimental procedures

Preparation of streptococcal genomic DNA. 50 ml Todd-Hewitt Broth medium was inoculated with *S. agalactiae* ATCC 12403 bacteria from a frozen stab and grown with aeration and shaking for 18 h at 37°C. The culture was then harvested, centrifuged with 1,600x g for 15 min and the supernatant was removed. Bacterial pellets were washed 3 x with PBS and carefully re-suspended in 0.5 ml of Lysozyme solution (100 mg/ml). 0.1 ml of 10 mg/ml heat treated RNase A and 20 U of RNase T1 were added, mixed carefully and the solution was incubated for 1 h at 37°C. Following the addition of 0.2 ml of 20 % SDS solution and 0.1 ml of Proteinase K (10 mg/ml) the tube was incubated overnight at 55°C. 1/3 volume of saturated NaCl was then added and the solution was incubated for 20 min at 4°C. The extract was pelleted in a microfuge (13,000 rpm) and the supernatant transferred into a new tube. The solution was extracted with PhOH/$CHCl_3$/IAA (25:24:1) and with $CHCl_3$/IAA (24:1). DNA was precipitated at room temperature by adding 0.6x volume of Isopropanol, spooled from the solution with a sterile Pasteur pipette and transferred into tubes containing 80% ice-cold ethanol. DNA was recovered by centrifuging the precipitates with 10-12,000x g, then dried on air and dissolved in ddH_2O.

Preparation of small genomic DNA fragments. Genomic DNA fragments were mechanically sheared into fragments ranging in size between 150 and 300 bp using a cup-horn sonicator (Bandelin Sonoplus UV

2200 sonicator equipped with a BB5 cup horn, 10 sec. pulses at 100 % power output) or into fragments of size between 50 and 70 bp by mild DNase I treatment (Novagen). It was observed that sonication yielded a much tighter fragment size distribution when breaking the DNA into fragments of the 150-300 bp size range. However, despite extensive exposure of the DNA to ultrasonic wave-induced hydromechanical shearing force, subsequent decrease in fragment size could not be efficiently and reproducibly achieved. Therefore, fragments of 50 to 70 bp in size were obtained by mild DNase I treatment using Novagen's shotgun cleavage kit. A 1:20 dilution of DNase I provided with the kit was prepared and the digestion was performed in the presence of $MnCl_2$ in a 60 µl volume at 20°C for 5 min to ensure double-stranded cleavage by the enzyme. Reactions were stopped with 2 µl of 0.5 M EDTA and the fragmentation efficiency was evaluated on a 2% TAE-agarose gel. This treatment resulted in total fragmentation of genomic DNA into near 50-70 bp fragments. Fragments were then blunt-ended twice using T4 DNA Polymerase in the presence of 100 µM each of dNTPs to ensure efficient flushing of the ends. Fragments were used immediately in ligation reactions or frozen at -20°C for subsequent use.

Description of the vectors. The vector pMAL4.31 was constructed on a pASK-IBA backbone {Skerra, A., 1994} with the beta-lactamase (*bla*) gene exchanged with the Kanamycin resistance gene. In addition the *bla* gene was cloned into the multiple cloning site. The sequence encoding mature beta-lactamase is preceded by the leader peptide sequence of *ompA* to allow efficient secretion across the cytoplasmic membrane. Furthermore a sequence encoding the first 12 amino acids (spacer sequence) of mature beta-lactamase follows the *ompA* leader peptide sequence to avoid fusion of sequences immediately after the leader peptidase cleavage site, since e.g. clusters of positive charged amino acids in this region would decrease or abolish translocation across the cytoplasmic membrane {Kajava, A. et al., 2000}. A *Sma*I restriction site serves for library insertion. An upstream FseI site and a downstream NotI site, which were used for recovery of the selected fragment, flank the *Sma*I site. The three restriction sites are inserted after the sequence encoding the 12 amino acid spacer sequence in such a way that the *bla* gene is transcribed in the -1 reading frame resulting in a stop codon 15 bp after the *Not*I site. A +1 bp insertion restores the *bla* ORF so that beta-lactamase protein is produced with a consequent gain of Ampicillin resistance.

The vector pMAL9.1 was constructed by cloning the *lamB* gene into the multiple cloning site of pEH1 {Hashemzadeh-Bonehi, L. et al., 1998}. Subsequently, a sequence was inserted in *lamB* after amino acid 154, containing the restriction sites *Fse*I, *Sma*I and *Not*I. The reading frame for this insertion was constructed in such a way that transfer of frame-selected DNA fragments excised by digestion with *Fse*I and *Not*I from plasmid pMAL4.31 yields a continuous reading frame of *lamB* and the respective insert.

The vector pHIE11 was constructed by cloning the *fhuA* gene into the multiple cloning site of pEH1. Thereafter, a sequence was inserted in *fhuA* after amino acid 405, containing the restriction site *Fse*I, *Xba*I and *Not*I. The reading frame for this insertion was chosen in a way that transfer of frame-selected DNA fragments excised by digestion with *Fse*I and *Not*I from plasmid pMAL4.31 yields a continuous reading frame of *fhuA* and the respective insert.

Cloning and evaluation of the library for frame selection. Genomic *S. agalactiae* DNA fragments were ligated into the *Sma*I site of the vector pMAL4.31. Recombinant DNA was electroporated into DH10B electrocompetent *E. coli* cells (GIBCO BRL) and transformants plated on LB-agar supplemented with Kanamycin (50 µg/ml) and Ampicillin (50 µg/ml). Plates were incubated over night at 37°C and colonies collected for large scale DNA extraction. A representative plate was stored and saved for collecting colonies for colony PCR analysis and large-scale sequencing. A simple colony PCR assay was used to initially determine the rough fragment size distribution as well as insertion efficiency. From sequencing data the precise fragment size was evaluated, junction intactness at the insertion site as well as the frame selection accuracy (*3n+1 rule*).

Cloning and evaluation of the library for bacterial surface display. Genomic DNA fragments were excised from the pMAL4.31 vector, containing the *S. agalactiae* library with the restriction enzymes *Fse*I and *Not*I. The

entire population of fragments was then transferred into plasmids pMAL9.1 (LamB) or pHIE11 (FhuA), which have been digested with *Fse*I and *Not*I. Using these two restriction enzymes, which recognise an 8 bp GC rich sequence, the reading frame that was selected in the pMAL4.31 vector is maintained in each of the platform vectors. The plasmid library was then transformed into *E. coli* DH5alpha cells by electroporation. Cells were plated onto large LB-agar plates supplemented with 50 μg/ml Kanamycin and grown over night at 37°C at a density yielding clearly visible single colonies. Cells were then scraped off the surface of these plates, washed with fresh LB medium and stored in aliquots for library screening at -80°C.

Results

Libraries for frame selection. Two libraries (LSAg-70 and LSAg-300) were generated in the pMAL4.31 vector with sizes of approximately 70 and 300 bp, respectively. For each library, ligation and subsequent transformation of approximately 1 μg of pMAL4.31 plasmid DNA and 50 ng of fragmented genomic *S. agalactiae* DNA yielded 4x 10^5 to 2x 10^6 clones after frame selection. To assess the randomness of the libraries, approximately 576 randomly chosen clones of LSAg-70 were sequenced. The bioinformatic analysis showed that of these clones only very few were present more than once. Furthermore, it was shown that approximately 80% of the clones fell in the size range between 25 and 100 bp with an average size of approximately 40 bp (Figure 2). Allmost all sequences followed the 3n+1 rule, showing that all clones were properly frame selected.

Bacterial surface display libraries. The display of peptides on the surface of *E. coli* required the transfer of the inserts from the LSAg-70 and LSAg-300 libraries from the frame selection vector pMAL4.31 to the display plasmids pMAL9.1 (LamB) or pHIE11 (FhuA). Genomic DNA fragments were excised by *Fse*I and *Not*I restriction and ligation of 5ng inserts with 0.1μg plasmid DNA and subsequent transformation into DH5alpha cells resulted in 2-5x 10^6 clones. The clones were scraped off the LB plates and frozen without further amplification.

Example 3: Identification of highly immunogenic peptide sequences from *S. agalactiae* using bacterial surface displayed genomic libraries and human serum

Experimental procedures

MACS screening. Approximately 2.5x 10^8 cells from a given library were grown in 5 ml LB-medium supplemented with 50 μg/ml Kanamycin for 2 h at 37°C. Expression was induced by the addition of 1 mM IPTG for 30 min. Cells were washed twice with fresh LB medium and approximately 2x 10^7 cells re-suspended in 100 μl LB medium and transferred to an Eppendorf tube.

10 to 20 μg of biotinylated, human IgGs purified from serum was added to the cells and the suspension incubated overnight at 4°C with gentle shaking. 900 μl of LB medium was added, the suspension mixed and subsequently centrifuged for 10 min at 6,000 rpm at 4°C (For IgA screens, 10 μg of purified IgAs were used and these captured with biotinylated anti-human-IgG secondary antibodies). Cells were washed once with 1 ml LB and then re-suspended in 100 μl LB medium. 10 μl of MACS microbeads coupled to streptavidin (Miltenyi Biotech, Germany) were added and the incubation continued for 20 min at 4°C. Thereafter 900 μl of LB medium was added and the MACS microbead cell suspension was loaded onto the equilibrated MS column (Miltenyi Biotech, Germany) which was fixed to the magnet. (The MS columns were equilibrated by washing once with 1 ml 70% EtOH and twice with 2 ml LB medium.)

The column was then washed three times with 3 ml LB medium. After removal of the magnet, cells were eluted by washing with 2 ml LB medium. After washing the column with 3 ml LB medium, the 2 ml eluate was loaded a second time on the same column and the washing and elution process repeated. The loading, washing and elution process was performed a third time, resulting in a final eluate of 2 ml.

A second and third round of screening was performed as follows. The cells from the final eluate were collected by centrifugation and re-suspended in 1 ml LB medium supplemented with 50 μg/ml Kanamycin. The culture was incubated at 37°C for 90 min and then induced with 1 mM IPTG for 30 min. Cells were subsequently collected, washed once with 1 ml LB medium and suspended in 10 μl LB medium. 10 to 20 μg of human, biotinylated IgGs were added again and the suspension incubated over night at 4°C with gentle shaking. All further steps were exactly the same as in the first selection round. Cells selected after two rounds of selection were plated onto LB-agar plates supplemented with 50 μg/ml Kanamycin and grown over night at 37°C.

Evaluation of selected clones by sequencing and Western blot analysis. Selected clones were grown overnight at 37°C in 3 ml LB medium supplemented with 50 μg/ml Kanamycin to prepare plasmid DNA using standard procedures. Sequencing was performed at MWG (Germany) or in collaboration with TIGR (U.S.A.).

For Western blot analysis approximately 10 to 20 μg of total cellular protein was separated by 10% SDS-PAGE and blotted onto HybondC membrane (Amersham Pharmacia Biotech, England). The LamB or FhuA fusion proteins were detected using human serum as the primary antibody at a dilution of approximately 1:3,000 to 1:5,000 and anti-human IgG or IgA antibodies coupled to HRP at a dilution of 1:5,000 as secondary antibodies. Detection was performed using the ECL detection kit (Amersham Pharmacia Biotech, England). Alternatively, rabbit anti-FhuA or rabbit anti-LamB polyclonal immune sera were used as primary antibodies in combination with the respective secondary antibodies coupled to HRP for the detection of the fusion proteins.

Results

Screening of bacterial surface display libraries by magnetic activated cell sorting (MACS) using biotinylated Igs. The libraries LSag-70 in pMAL9.1 and LSag-300 in pHIE11 were screened with pools of biotinylated, human IgGs and IgAs prepared from sera of healthy adults (NSag8-IgG,-IgA) or P10,11,18 (see Example 1: *Preparation of antibodies from human serum*). The selection procedure was performed as described under Experimental procedures. Figure 3A shows a representative example of a screen with the LSag-70 library and PSag11-IgGs. As can be seen from the colony count after the first selection cycle from MACS screening, the total number of cells recovered at the end is drastically reduced from $2x10^7$ cells to approximately $2x\ 10^4$ cells, but the selection without antibodies added showed a similar reduction in cell numbers (Figure 3A). Therefore a second and third round of selection was performed. At the end of round three, approximately 10^4 cells was recovered with PSag11-IgGs, while only $2x\ 10^3$ cells were recovered when no IgGs from human serum were added, clearly showing that selection was dependent on *S. agalactiae* specific antibodies. To evaluate the performance of the screen, 26 selected clones were picked randomly and subjected to immunoblot analysis with screening IgG pool (Figure 3B). This analysis revealed that more than 80% of the selected clones showed reactivity with antibodies present in the relevant serum whereas the control strain expressing LamB without a *S. agalactiae* specific insert did not react with the same serum. In general, the rate of reactivity was observed to lie within the range of 35 to 90%. Colony PCR analysis showed that all selected clones contained an insert in the expected size range.

Subsequent sequencing of a larger number of randomly picked clones (600 to 1200 per screen) led to the identification of the gene and the corresponding peptide or protein sequence that was specifically recognized by the human serum antibodies used for screening. The frequency with which a specific clone is selected reflects at least in part the abundance and/or affinity of the specific antibodies in the serum used for selection and recognizing the epitope presented by this clone. In that regard it is striking that clones derived from some ORFs (e.g. gbs1087, gbs1306, gbs2018) were picked more than 100 times, indicating their highly immunogenic property. Table 1 and Table 5 summarizes the data obtained for all

13 performed screens. All clones that are presented in Table 1 and Table 5 have been verified by immunoblot analysis using whole cellular extracts from single clones to show the indicated reactivity with the pool of human serum used in the respective screen. As can be seen from Table 1 and Table 5, distinct regions of the identified ORF are identified as immunogenic, since variably sized fragments of the proteins are displayed on the surface by the platform proteins.

It is further worth noticing that most of the genes identified by the bacterial surface display screen encode proteins that are either attached to the surface of *S. agalactiae* and/or are secreted. This is in accordance with the expected role of surface attached or secreted proteins in virulence of *S. agalactiae*.

Example 4: Assessment of the reactivity of highly immunogenic peptide sequences with individual human sera.

Experimental procedures

Peptide synthesis
Peptides were synthesized in small scale (4 mg resin; up to 288 in parallel) using standard F-moc chemistry on a Rink amide resin (PepChem, Tübingen, Germany) using a SyroII synthesizer (Multisyntech, Witten, Germany). After the sequence was assembled, peptides were elongated with Fmoc-epsilon-aminohexanoic acid (as a linker) and biotin (Sigma, St. Louis, MO; activated like a normal amino acid). Peptides were cleaved off the resin with 93%TFA, 5% triethylsilane, and 2% water for one hour. Peptides were dried under vacuum and freeze dried three times from acetonitrile/water (1:1). The presence of the correct mass was verified by mass spectrometry on a Reflex III MALDI-TOF (Bruker, Bremen Germany). The peptides were used without further purification.

Enzyme linked immune assay (ELISA).
Biotin-labeled peptides (at the N-terminus) were coated on Streptavidin ELISA plates (EXICON) at 10 µg/ml concentration according to the manufacturer's instructions. Highly specific Horse Radish Peroxidase (HRP)-conjugated anti-human IgG secondary antibodies (Southern Biotech) were used according to the manufacturers' recommendations (dilution: 1,000x). Sera were tested at two serum dilutions, 200X and 1,000X. Following manual coating, peptide plates were processed and analyzed by the Gemini 160 ELISA robot (TECAN) with a built-in ELISA reader (GENIOS, TECAN).

Results

Following the bioinformatic analysis of selected clones, corresponding peptides were designed and synthesized. In case of epitopes with more than 26 amino acid residues, overlapping peptides were made. All peptides were synthesized with a N-terminal biotin-tag and used as coating reagents on Streptavidin-coated ELISA plates.

The analysis was performed with peptides that were selected based on their reactivity with the individual sera, which were included in the serum pools used for preparations of IgG and IgA screening reagents for bacterial surface display. A summary for serum reactivity of 74 peptides representing 55 different *S. agalactiae* antigenic proteins from the genomic screen analysed with 22 human sera (from 13 patient and 9 healthy exposed high titer individuals) used for the antigen identification is shown in Table 2 and Table 6. The peptides were compared by the score calculated for each peptide based on the number of positive sera and the extent of reactivity. Peptides range from highly and widely reactive to weakly positive ones.

Example 5: Gene distribution studies with highly immunogenic proteins identified from *S. agalactiae*.

Experimental procedures

Gene distribution of GBS antigens by PCR. An ideal vaccine antigen would be an antigen that is present in all, or the vast majority of strains of the target organism to which the vaccine is directed. In order to establish whether the genes encoding the identified *Streptococcus agalactiae* antigens occur ubiquitously in *S. agalactiae* strains, PCR was performed on a series of independent *S. agalactiae* isolates with primers specific for the gene of interest. *S. agalactiae* isolates were obtained covering the serotypes most frequently present in patients as shown in Figure 4A. Oligonucleotide sequences as primers were designed for all identified ORFs yielding products of approximately 1,000 bp, if possible covering all identified immunogenic epitopes. Genomic DNA of all *S. agalactiae* strains was prepared as described under Example 2. PCR was performed in a reaction volume of 25 µl using Taq polymerase (1U), 200 nM dNTPs, 10 pMol of each oligonucleotide and the kit according to the manufacturers instructions (Invitrogen, The Netherlands). As standard, 30 cycles (1x: 5min. 95°C, 30x: 30sec. 95°C, 30sec. 56°C, 30sec. 72°C, 1x 4min. 72°C) were performed, unless conditions had to be adapted for individual primer pairs.

Results

Identified genes encoding immunogenic proteins were tested by PCR for their presence in 46 different strains of *S. agalactiae* (Figure 4A). As an example, figure 4B shows the PCR reaction for GBS0061 with all indicated 46 strains. As clearly visible, the gene is present in all strains analysed. The PCR fragment from a type IA strain was sequenced and showed that all 657 bp were identical as compared to the *S. agalactiae* ATCC 12403 strain, indicating a high level of conservation between the two isolates.
From a total of 117 genes analysed, more than 100 were present in all or almost all strains tested, while only 5 genes were absent in more than 10% of the tested 46 strains (Table 3). In addition, only few genes (e.g. GBS0016, GBS1087, GBS1528 and GBS2018) showed variation in size but were present in all or most strain isolates. Sequencing of the generated PCR fragment from one strain and subsequent comparison to the type III strain ATCC 12403 confirmed the amplification of the correct DNA fragment and revealed a degree of sequence divergence as indicated in Table 3. Importantly, many of the identified antigens are well conserved in all strains in sequence and size and are therefore novel vaccine candidates to prevent infections by GBS.

Example 6: Characterization of immune sera obtained from mice immunized with highly immunogenic proteins/peptides from *S. agalactiae* displayed on the surface of *E. coli*.

Experimental procedures

Generation of immune sera from mice
E. coli clones harboring plasmids encoding the platform protein fused to a *S. agalactiae* peptide, were grown in LB medium supplemented with 50µg/ml Kanamycin at 37°C. Overnight cultures were diluted 1:10, grown until an OD_{600} of 0.5 and induced with 0.2 mM IPTG for 2 hours. Pelleted bacterial cells were suspended in PBS buffer and disrupted by sonication on ice, generating a crude cell extract. According to the OD_{600} measurement, an aliquot corresponding to $5x10^7$ cells was injected into NMRI mice i.v., followed by a boost after 2 weeks. Serum was taken 1 week after the second injection. Epitope specific antibody levels were measured by peptide ELISA.

In vitro expression of antigens
Expression of antigens by *in vitro* grown *S. agalactiae* serotype III was tested by immunoblotting. Different growth media and culture conditions were tested to detect the presence of antigens in total lysates and bacterial culture supernatants. Expression was considered confirmed when a specific band corresponding to the predicted molecular weight and electrophoretic mobility was detected.

Cell surface staining
Flow cytometric analysis was carried out as follows. Bacteria were grown under culture conditions, which resulted in expression of the antigen as shown by the immunoblot analysis. Cells were washed twice in Hanks Balanced Salt Solution (HBSS) and the cell density was adjusted to approximately 1 X 10^6 CFU in 100µl HBSS, 0.5% BSA. After incubation for 30 to 60 min at 4°C with mouse antisera diluted 50 to 100-fold, unbound antibodies were washed away by centrifugation in excess HBSS, 0.5% BSA. Secondary goat anti-mouse antibody ($F(ab')_2$ fragment specific) labeled with fluorescein (FITC) was incubated with the cells at 4°C for 30 to 60 min. After washing, cells were fixed with 2% paraformaldehyde. Bound antibodies were detected using a Becton Dickinson FACScan flow cytometer and data further analyzed with the computer program CELLQuest. Negative control sera included mouse pre-immune serum and mouse polyclonal serum generated with lysates prepared from IPTG induced *E. coli* cells transformed with plasmids encoding the genes *lamB* or *fhuA* without *S. agalactiae* genomic insert.

Bactericidal (killing) assay
Murine macrophage cells (RAW246.7 or P388.D1) and bacteria were incubated and the loss of viable bacteria after 60 min was determined by colony counting. In brief, bacteria were washed twice in Hanks Balanced Salt Solution (HBSS) and the cell density was adjusted to approximately 1X 10^5 CFU in 50µl HBSS. Bacteria were incubated with mouse sera (up to 25%) and guinea pig complement (up to 5%) in a total volume of 100µl for 60min at 4°C. Pre-opsonized bacteria were mixed with macrophages (murine cell line RAW264.7 or P388.D1; 2X 10^6 cells per 100µl) at a 1:20 ratio and were incubated at 37°C on a rotating shaker at 500 rpm. An aliquot of each sample was diluted in sterile water and incubated for 5 min at room temperature to lyse macrophages. Serial dilutions were then plated onto Todd-Hewitt Broth agar plates. The plates were incubated overnight at 37°C, and the colonies were counted with the Countermat flash colony counter (IUL Instruments). Control sera included mouse pre-immune serum and mouse polyclonal serum generated with lysates prepared from IPTG induced *E. coli* transformed with plasmids harboring the genes *lamB* or *fhuA* without *S. agalactiae* genomic insert.

Results

Immunogenicity in mice. The presence of specific antibodies was determined by peptide ELISA and/or immunoblotting using the *E. coli* clone expressing the given epitope embedded in LamB or FhuA platform proteins, as it is exemplified in Fig. 5A and B, respectively, and summarized in Table 4 and Table 7. 43 novel GBS antigens represented by 61 different epitope regions were shown to be immunogenic in mice. Positive sera were then analysed by immunblotting using total bacterial lysates and culture supernatants prepared from *S. agalactiae* serotype III strain (data not shown). This analysis served as a first step to determine whether the antigenic proteins were expressed, and if, under which growth conditions, in order to evaluate surface expression of the polypeptide by FACS analysis. It was anticipated based on literature data that not all proteins would be expressed under *in vitro* conditions.

Cell surface staining of S. agalactiae. Cell surface accessibility for several antigenic proteins was subsequently demonstrated by an assay based on flow cytometry. GBS cells were incubated with preimmune and polyclonal mouse sera raised against *S. agalactiae* lysate or *E. coli* clones harboring plasmids encoding the platform protein fused to a *S. agalactiae* peptide, followed by detection with fluorescently tagged secondary antibody. As shown in Fig. 6A, antisera raised against *S. agalactiae* lysate contains antibodies against surface components, demonstrated by a significant shift in fluorescence of the *S. agalactiae* serotype III cell population. Similar cell surface staining of *S. agalactiae* serotype III cells was observed with polyclonal sera raised against peptides of many of the GBS antigens identified (Fig. 6B). In some instancies, a subpopulation of the bacteria was not stained, as indicated by the detection of two peaks in the histograms (Fig. 6B). This phenomenon may be a result of differential expression of the gene products during the growth of the bacterium, insufficient antibody levels or partial inhibition of antibody binding caused by other surface molecules or plasma proteins. Importantly, a well-known protective GBS

antigen, Sip/gbs0031 is proved to be also positive in this assay.

In vitro bactericidal activity. Opsonophagocytic killing is the cornerstone of host defense against extracellular bacteria, such as *S. agalactiae.* Cell surface binding of antibodies to bacterial antigens are opsonizing and induce killing (bactericidal) by phagocytic cells (macrophages and neutrophil granulocytes) if the antibodies induced by the particular antigens can bind activated complement components (C3bi). In Figure 7 data are presented on bactericidal activity measured by antigen-specific antibodies generated in mice with corresponding epitopes. According to these data, several of the novel GBS antigens, for example gbs0012, gbs0016, gbs0428, gbs1306 and gbs2018 induce functional antibodies. Importantly, a well-known protective GBS antigen, Sip/gbs0031 is proved to be strongly positive in the very same assay.

These experiments confirmed the bioinformatic prediction that many of the proteins are exported due to their signal peptide sequence and in addition showed that they are present on the cell surface of *S. agalactiae* serotype III. They also confirm that these proteins are available for recognition by human antibodies with functional properties and make them valuable candidates for the development of a vaccine against GBS diseases.

Table 1A: Immunogenic proteins identified by bacterial surface display.

S. agalactiae antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immunogenic region (aa)	Seq. ID (DNA, Prot.)
gbs0012	weakly similar to beta-lactamase	4-20,35-44,65-70,73-87,92-98,112-137,152-161,177-186,193-200,206-213,229-255,282-294,308-313,320-326,349-355,373-384,388-406,420-425	B:13, C:6	115-199	1, 218
gbs0016	glucan-binding protein B	5-24,35-41,44-70,73-89,103-109,127-143,155-161,185-190,192-207,212-219,246-262,304-336,372-382,384-393,398-407,412-418,438-444	B:12, C:4, D:3, E:5, H:4, I:12, M:2	1-75, 76-161, 164-239	2, 219
gbs0024	phosphoribosylformyl glycinamidine synthase	4-10,16-58,60-71,77-92,100-126,132-146,149-164,166-172,190-209,214-220,223-229,241-256,297-312,314-319,337-343,351-359,378-387,398-418,421-428,430-437,440-448,462-471,510-519,525-536,552-559,561-568,573-582,596-602,608-630,637-649,651-665,681-702,714-732,739-745,757-778,790-805,807-815,821-829,836-842,846-873,880-903,908-914,916-923,931-940,943-948,956-970,975-986,996-1015,1031-1040,1051-1069,1072-1095,1114-1119,1130-1148,1150-1157,1169-1176,1229-1238	F:4	802-812	3, 220
gbs0031	surface immunogenic protein	5-12,14-26,35-47,52-67,72-78,83-98,121-141,152-159,163-183,186-207,209-257,264-277,282-299,301-309,312-318,324-339,358-368,372-378,387-397,425-431	A:17, B:53, C:36, D:4	46-291	4, 221
gbs0048	Hypothetical protein	29-38,44-64,70-76,78-87,94-100,102-112,119-134,140-149,163-173,178-186,188-194,207-234,247-262,269-290	K:13	73-92	5, 222
gbs0053	aldehyde-alcohol dehydrogenase (adhE)	10-28,36-63,77-87,103-119,127-136,141-169,171-183,195-200,207-232,236-246,251-265,268-283,287-297,314-322,335-343,354-363,384-390,405-411,419-436,443-455,467-473,480-513,518-529,550-557,565-585,602-608,616-625,632-660,665-677,685-701,726-736,738-747,752-761,785-796,801-813,838-853,866-871	E:4	757-774	6, 223
gbs0061	rplB ribosomal protein L2	31-38,61-66,74-81,90-115,123-145,154-167,169-179,182-193,200-206,238-244,267-272	F:2, I:12	235-251	7, 224
gbs0084	DNA-directed RNA polymerase, alpha subunit (rpoA)	19-25,38-54,56-64,66-72,74-92,94-100,116-129,143-149,156-183,204-232,253-266,269-275,294-307	C:4, D:6	241-313	8, 225
gbs0107	conserved hypothetical protein	5-34,50-56,60-65,74-85,89-97,108-119,159-165,181-199,209-225,230-240,245-251,257-262,274-282,300-305	K:2	64-75	9, 226

S. agalactiae antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immunogenic region (aa)	Seq. ID (DNA, Prot.)
gbs0108	deoxyuridine 5`-triphosphate nucleotidohydrolase	5-13,16-21,27-42,45-52,58-66,74-87,108-114,119-131	I:5	39-51	10, 227
gbs0113	ribose ABC transporter	6-23,46-54,59-65,78-84,100-120,128-133,140-146,159-165,171-183,190-204,224-232,240-248,250-259,274-280,288-296,306-315	F:4	267-274	11, 228
gbs0123	similar to argininosuccinate synthase	5-12,15-24,26-36,42-65,68-80,82-104,111-116,125-144,159-167,184-189,209-218,235-243,254-265,269-283,287-300,306-316,318-336,338-352,374-392	K:17	162-174	12, 229
gbs0127	rpmV 50S ribosomal protein L28	30-42,45-54	F:11	25-37	13, 230
gbs0144	oligopeptide ABC transporter, substrate-binding	10-30,53-59,86-95,116-130,132-147,169-189,195-201,212-221,247-256,258-265,278-283,291-298,310-316,329-339,341-352,360-367,388-396,398-411,416-432,443-452,460-466,506-512,515-521,542-548	E:7	419-431	14, 231
gbs0183	membrane protein, putative	4-27,30-53,60-67,70-90,92-151,159-185,189-195,198-210,215-239	F:9	173-189	15, 232
gbs0184	oligopeptide ABC transporter, oligopeptide-binding	4-26,41-54,71-78,116-127,140-149,151-158,161-175,190-196,201-208,220-226,240-252,266-281,298-305,308-318,321-329,344-353,372-378,384-405,418-426,429-442,457-463,494-505,514-522	E:6	174-188	16, 233
gbs0235	glycine betaine/carnitine/choline ABC transporter	17-25,27-39,61-67,81-89,99-110,120-131,133-139,147-161,167-172,179-185,192-198,203-213,226-238,243-258,261-267,284-290,296-307,311-328,340-352,356-371	G:8, H:15	239-256	17, 234
gbs0255	conserved hypothetical protein	8-30,40-49,67-80,114-123,126-142,152-162,188-194	E:2	57-70	18, 235
gbs0260	glycyl-tRNA synthetase (beta subunit)	4-23,28-34,36-47,50-61,76-81,89-94,96-104,112-119,126-146,155-181,195-200,208-214,220-229,244-260,263-276,282-288,292-300,317-323,336-351,353-359,363-375,382-399,415-432,444-455,458-471,476-481,484-492,499-517,522-529,535-541,543-568,572-584,586-600,607-617,626-637,656-675	F:3	282-297	19, 236
gbs0268	transketolase (tkt)	6-24,30-35,38-45,63-91,134-140,146-160,167-188,214-220,226-234,244-250,260-270,286-301,316-329,340-371,429-446,448-459,474-481,485-491,512-526,537-544,550-565,573-583,596-613,621-630,652-658	E:7	87-97	20, 237
gbs0286	NH3-dependent	8-20,26-48,56-67,76-86,94-109,115-121,123-129,143-	F:12, G:7, H:8	237-247	21, 238

S. agalactiae antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immunogenic region (aa)	Seq. ID (DNA, Prot.)
	NAD+ synthetase	160,178-186,191-198,201-208,221-236,238-244,260-268			
gbs0288	similar to penicillin-binding protein 1A	4-40,42-57,73-87,98-117,126-135,150-156,166-174,196-217,231-236,248-258,276-284,293-301,307-313,339-347,359-365,375-387,395-402,428-440,445-456,485-490,497-505,535-541,547-555,610-625,648-656,665-671	D:5, K:3	448-528	22, 239
gbs0343	seryl-tRNA synthetase (serS)	10-18,39-45,51-61,80-96,98-106,110-115,158-172,174-183,191-200,220-237,249-255,274-289,308-324,331-341,372-381,384-397,405-414	I:3	322-338	23, 240
gbs0411	Hypothetical protein	30-36,38-56,85-108,134-147,149-160,163-183,188-201,206-211,219-238,247-254	I:11	5-13	24, 241
gbs0428	similar to fibrinogen binding protein, putative peptidoglycan linked protein (LPXTG motif)	11-40,98-103,110-115,133-145,151-159,172-179,192-201,204-212,222-228,235-245,258-268,283-296,298-309,322-329,342-351,354-362,372-378,385-393,407-418,495-516	A:7, B:2, C:31	1-148	25, 242
gbs0437	glucose-6-phosphate isomerase (pgi)	5-19,21-36,73-94,112-119,122-137,139-145,152-167,184-190,198-204,208-224,249-265,267-281,299-304,309-317,326-333,356-364,368-374,381-389,391-414,419-425,430-435	I:26	113-140	26, 243
gbs0460	decarboxylase	45-54,59-67,78-91	I:7, K:11	15-23	27, 244
gbs0465	oxydoreductase	11-22,33-47,52-80,88-112,124-129	F:4	6-25	28, 245
gbs0470	similar to alpha protein, putative peptidoglycan linked protein (LPXTG motif)	26-41,51-63,80-89,93-115,150-163,187-193,220-237,240-249,286-294,296-306,316-329,345-353,361-370,407-425,428-437,474-482,484-494,504-517,533-541,549-558,595-613,616-625,660-668,673-685,711-726,736-744,749-761,787-802,812-820,825-837,863-878,888-896,901-913,939-954,964-972,977-989,1003-1008,1016-1022,1028-1034,1041-1053,1059-1074,1101-1122	B:4, C:2, D:8	420-511, 581-704	29, 246
gbs0489	acetyltransferase, GNAT family	18-25,27-55,71-83,89-95,102-113,120-146,150-156,174-185	E:32	159-175	30, 247
gbs0492	gbs0492 valyl-tRNA synthetase	24-30,38-56,63-68,87-93,136-142,153-164,183-199,213-219,226-234,244-261,269-278,283-289,291-297,320-328,330-336,340-346,348-356,358-366,382-387,401-408,414-419,449-455,468-491,504-512,531-537,554-560,597-608,621-627,632-643,650-662,667-692,703-716,724-737,743-758,783-794,800-818,846-856	A:3	806-884	31, 248

S. agalactiae antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immunogenic region (aa)	Seq. ID (DNA, Prot.)
gbs0538	amino acid ABC transporter (ATP-binding protein)	4-14,21-39,86-92,99-107,121-131,136-144,147-154,158-166,176-185,193-199,207-222,224-230	G:1	117-136	32, 249
gbs0539	similar to phosphomannomutase	65-76,85-97,103-109,115-121,125-146,163-169,196-205,212-219,228-237,241-247,254-262,269-288,294-303,305-313,328-367,395-401,405-412,418-429,437-447,481-488,506-513,519-524,530-541,546-557	K:4	266-284	33, 250
gbs0555	beta-lactam resistance factor (fibA)	5-14,37-42,49-71,78-92,97-112,127-136,147-154,156-163,186-198,216-225,233-243,248-253,295-307,323-332,359-366,368-374,380-398	E:3	194-223	34, 251
gbs0579	dipeptidase	4-11,33-39,45-72,100-113,119-129,136-144,169-175,177-185,200-208,210-219,262-276,278-297,320-326,336-344,347-362,381-394,443-453	I:4	438-454	35, 252
gbs0580	zinc ABC transporter, zinc-binding adhesion, lipoprotein	4-29,31-52,55-61,95-110,138-158,162-171,179-187,202-229,239-248,251-256,262-267,269-285,304-310,351-360,362-368,381-388,415-428,435-440,448-458	I:11	161-178	36, 253
gbs0628	cell wall surface anchor family protein (IPxTG)	4-17,19-28,32-43,47-59,89-110,112-126,128-134,140-148,152-161,169-184,191-204,230-235,255-264,328-338,341-347,401-409,413-419,433-441,449-458,463-468,476-482,486-492,500-506,529-545	I:9, H:1	305-381	37, 254
gbs0632	cell wall surface anchor family protein, putative (FPKTG motive)	10-29,38-45,53-61,134-145,152-160,163-170,202-208,219-229,248-258,266-275,282-288,315-320,328-334,377-385,392-402,418-424,447-453,460-471,479-487,491-497,500-507,531-537,581-594,615-623,629-635,644-652,659-666,668-678,710-717,719-728,736-741,747-760,766-773,784-789,794-800,805-817,855-861,866-887	H:3	698-715	38, 255
gbs0634	putative surface protein	16-26,29-37,44-58,62-68,74-80,88-95,97-120,125-144,165-196	H:1	58-72	39, 256
gbs0667	regulatory protein, putative, truncation	14-21,23-46,49-60,63-74,78-92,96-103,117-129,134-161,169-211,217-231,239-248,252-281,292-299,313-343	I:2	243-257	40, 257
gbs0672	transcriptional regulator (GntR family)	11-27,46-52,67-72,76-84,91-112,116-153,160-175,187-196,202-211,213-220	G:11	43-76	41, 258
gbs0687	Hypothetical protein	5-29,37-56,78-86,108-118,152-161	I:4	120-130	42, 259
gbs0785	Similar to penicillin binding protein 2B	8-14,19-41,52-66,75-82,87-92,106-121,127-133,136-143,158-175,180-187,196-204,221-228,239-245,259-265,291-306,318-323,328-340,352-358,361-368,375-	K:3	184-196	43, 260

S. agalactiae antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immunogenic region (aa)	Seq. ID (DNA, Prot.)
		381,391-399,411-418,431-442,446-455,484-496,498-510,527-533,541-549,558-565,575-585,587-594,644-655,661-668,671-677			
gbs0811	beta-glucosidase	4-22,29-38,55-62,75-81,102-107,110-134,143-150,161-167,172-179,191-215,223-233,241-247,251-264,266-272,288-309,340-352,354-366,394-402,414-438	L:13	198-218	44, 261
gbs0828	hypothetical protein	24-44,49-70,80-91,105-118,128-136,140-154	I:3	77-92	45, 262
gbs0851	hypothetical protein	5-22,31-36,41-47,67-74,83-90,105-122,135-143,160-167	E:2	118-129	46, 263
gbs0865	hypothetical protein	4-25,33-73,81-93,96-106,114-120,122-128,130-172,179-208,210-241,251-283,296-301	K:4	92-100	47, 264
gbs0890	exonuclease RexB (rexB)	14-24,29-38,43-50,52-72,86-97,101-107,110-125,127-141,145-157,168-175,177-184,186-195,205-226,238-250,255-261,284-290,293-304,307-314,316-323,325-356,363-371,383-390,405-415,423-432,442-454,466-485,502-511,519-527,535-556,558-565,569-574,612-634,641-655,672-686,698-709,715-722,724-732,743-753,760-769,783-792,818-825,830-839,842-849,884-896,905-918,926-940,957-969,979-1007,1015-1021,1049-1057	E:5	336-349	48, 265
gbs0896	similar to acetoin dehydrogenase	6-16,26-31,33-39,62-73,75-85,87-100,113-123,127-152,157-164,168-181,191-198,208-214,219-226,233-254,259-266,286-329	K:2	181-195	49, 266
gbs0898	acetoin dehydrogenase, thymine PPi dependent	4-13,32-39,53-76,99-108,110-116,124-135,137-146,149-157,162-174,182-190,207-231,242-253,255-264,274-283,291-323,334-345,351-360,375-388,418-425,456-474,486-492,508-517,520-536,547-560,562-577	E:13, F:2, I:2, J:2	31-45, 419-443	50, 267
gbs0904	phosphoglucomutase /phosphomannomutase family protein	15-26,30-37,42-49,58-90,93-99,128-134,147-154,174-179,190-197,199-205,221-230,262-274,277-287,300-314,327-333,343-351,359-377,388-396,408-413,416-425,431-446	I:3	246-256	51, 268
gbs0918	weakly similar to histidine triad protein, putative lipoprotein	5-26,34-42,47-54,61-67,71-104,107-115,131-138,144-153,157-189,196-202,204-210,228-245,288-309,316-329,332-341,379-386,393-399,404-412,414-421,457-468,483-489,500-506,508-517,523-534,543-557,565-580,587-605,609-617,619-627,631-636,640-646,662-668,675-682,705-710,716-723,727-732,750-758,784-789,795-809,869-874	B:5, C:11, D:36, E:3, K:3	14-138, 166-286, 372-503, 674-696, 754-859	52, 269

S. agalactiae antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immunogenic region (aa)	Seq. ID (DNA, Prot.)
gbs0931	pyruvate kinase	5-17,32-38,40-47,80-89,113-119,125-137,140-154,157-163,170-177,185-199,213-225,228-236,242-248,277-290,292-305,323-333,347-353,364-370,385-394,399-406,423-433,441-451,462-474,477-487	F:78	116-124	53, 270
gbs0947	similar to L-Lactate Dehydrogenase	7-16,18-30,32-49,53-61,63-85,95-101,105-115,119-134,143-150,159-178,185-202,212-229,236-250,254-265,268-294	K:28	63-72	54, 271
gbs0948	DNA gyrase, A subunit (gyrA)	4-12,19-47,73-81,97-103,153-169,188-198,207-213,217-223,236-242,255-265,270-278,298-305,309-317,335-347,354-363,373-394,419-424,442-465,486-492,500-507,542-549,551-558,560-572,580-589,607-614,617-623,647-653,666-676,694-704,706-714,748-754,765-772,786-792,795-806	I:4	358-370	55, 272
gbs0969	similar to unknown plasmid protein	18-28,30-38,40-46,49-55,69-78,82-98,104-134,147-153,180-190,196-202,218-236,244-261,266-273,275-286,290-295,301-314,378-387,390-395,427-434	E:3	290-305	56, 273
gbs0971	similar to putative plasmid replication protein	4-13,20-31,39-51,54-61,69-84,87-105,117-124	K:17	108-125	57, 274
gbs0972	Hypothetical protein	24-34,43-54,56-66,68-79	E:3	50-69	58, 275
gbs0983	similar to plasmid protein	5-43,71-77,102-131,141-148,150-156,159-186,191-207,209-234,255-268,280-286,293-299,317-323,350-357,363-372,391-397,406-418,428-435,455-465,484-497,499-505,525-531,575-582,593-607,621-633,638-649,655-673,684-698,711-725,736-741,743-752,759-769,781-793,813-831,843-853,894-905,908-916,929-946,953-963,970-978,1001-1007,1011-1033	D:11, E:2, F:2, J:10, K:10, L:46, M:3	165-178, 818-974	59, 276
gbs0986	surface antigen proteins, putative peptidoglycan bound	16-44,63-86,98-108,185-191,222-237,261-274,282-294,335-345,349-362,374-384,409-420,424-430,440-447,453-460,465-473,475-504,522-534,538-551,554-560,567-582,598-607,611-619,627-640,643-653,655-661,669-680,684-690,701-707,715-731,744-750,756-763,768-804,829-837,845-853,855-879,884-890,910-928	B:3, C:12, D:2, E:3, F:20, H:3, I:3, J:5, M:2	77-90, 144-212, 279-355, 434-536, 782-810, 875-902	60, 277
gbs0988	similar to plasmid surface exclusion protein, putative peptidoglycan bound	4-22,29-41,45-51,53-66,70-77,86-95,98-104,106-124,129-135,142-151,153-161,169-176,228-251,284-299,331-337,339-370,380-387,393-398,406-411,423-433,440-452,461-469,488-498,501-516,523-530,532-559,562-	J:2	212-227	61, 278

S. agalactiae antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immunogenic region (aa)	Seq. ID (DNA, Prot.)
	protein (LPXTG motif)	567,570-602,612-628,630-645,649-659,666-672,677-696,714-723,727-747			
gbs0991	ATP-dependent Clp protease, ATP-binding subunit ClpA	4-9,17-31,35-41,56-61,66-75,81-87,90-124,133-138,149-163,173-192,213-219,221-262,265-275,277-282,292-298,301-307,333-346,353-363,371-378,419-430,435-448,456-469,551-570,583-599,603-612	F:15	275-291	62, 279
gbs0993	similar to plasmid proteins	28-34,53-58,72-81,100-128,145-154,159-168,172-189,217-225,227-249,256-263,299-309,322-330,361-379,381-388,392-401,404-417,425-436,440-446,451-464,469-487,502-511,543-551,559-564,595-601,606-612,615-626,633-642,644-650,664-670,674-684,692-701,715-723,726-734,749-756,763-771,781-787,810-843,860-869,882-889,907-917,931-936,941-948,951-958,964-971,976-993,1039-1049,1051-1065,1092-1121,1126-1132,1145-1151,1158-1173,1181-1192,1194-1208,1218-1223,1229-1243,1249-1254,1265-1279,1287-1297,1303-1320,1334-1341,1343-1358,1372-1382,1406-1417,1419-1425,1428-1434,1441-1448,1460-1473,1494-1504,1509-1514,1529-1550	B:2, F:2, J:4, K:2, M:7	654-669, 1400-1483	63, 280
gbs0995	hypothetical protein	10-16,20-25,58-65,97-109,118-132,134-146,148-155,186-195,226-233,244-262,275-284,295-310,317-322,330-339,345-351,366-375,392-403,408-415,423-430,435-444,446-457,467-479,486-499,503-510,525-537,540-585,602-612,614-623,625-634,639-645,650-669,700-707,717-724,727-739	H:3, I:39, J:3, M:3	205-230, 733-754	64, 281
gbs0997	hypothetical protein	5-22,37-43,72-81,105-113,128-133,148-160,188-194,204-230,238-245,251-257	D:2, F:52	194-213	65, 282
gbs0998	hypothetical protein	16-21,35-41,56-72,74-92,103-109	I:2	62-68	66, 283
gbs1001	hypothetical protein	4-15,17-82,90-104,107-159,163-170,188-221,234-245,252-265	G:8	220-235	67, 284
gbs1015	hypothetical protein	16-22,36-46,61-75,92-107,113-121,139-145,148-160	K:17	30-42	68, 285
gbs1035	conserved hypothetical protein	4-12,20-26,43-49,55-62,66-78,121-127,135-141,146-161,164-170,178-189,196-205,233-238,269-279,288-318,325-332,381-386,400-407	E:3	328-346	69, 286
gbs1041	hypothetical protein	5-12,31-49,57-63,69-79,89-97,99-114,116-127,134-142,147-154,160-173,185-193,199-204,211-222,229-236,243-249,256-274	L:2	58-68	70, 287

S. agalactiae antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immunogenic region (aa)	Seq. ID (DNA, Prot.)
gbs1066	hypothetical protein	10-20,28-34,39-53,68-79,84-90,99-106	K:2	73-79	71, 288
gbs1087	FbsA	14-37,45-50,61-66,77-82,93-98,109-114,125-130,141-146,157-162,173-178,189-194,205-210,221-226,237-242,253-258,269-274,285-290,301-306,316-332,349-359,371-378,385-406	A:7, B:2, C:4, E:277, G:523, J:25	34-307, 312-385	72, 289
gbs1103	ABC transporter (ATP-binding protein)	4-10,17-38,50-85,93-99,109-116,128-185,189-197,199-210,223-256,263-287,289-312,327-337,371-386,389-394,406-419,424-432,438-450,458-463,475-502,507-513,519-526,535-542,550-567	I:5	361-376	73, 290
gbs1116	xanthine permease (pbuX)	10-39,42-93,100-144,155-176,178-224,230-244,246-255,273-282,292-301,308-325,332-351,356-361,368-379,386-393,400-421	I:48	138-155	74, 291
gbs1126	similar to plasmid unknown protein	5-11,17-34,40-45,50-55,72-80,101-123,145-151,164-172,182-187,189-195,208-218,220-241,243-252,255-270,325-331,365-371,391-398,402-418,422-428,430-435,443-452,463-469,476-484,486-494,503-509,529-553,560-565,570-590,608-614,619-627,654-661,744-750,772-780,784-790,806-816,836-853,876-885,912-918,926-933,961-975,980-987,996-1006,1016-1028,1043-1053,1057-1062	E:2, K:3	994-1003, 1033-1056	75, 292
gbs1143	putative peptidoglycan linked protein (LPXTG)	17-45,64-71,73-81,99-109,186-192,223-238,262-275,283-295,336-346,350-363,375-385,410-421,425-431,441-448,454-463,468-474,476-512,523-537,539-552,568-583,599-608,612-620,628-641,644-654,656-662,670-681,685-695,702-708,716-723,725-735,757-764,769-798,800-806,808-816,826-840,846-854,856-862,874-881,885-902,907-928	C:3, D:2, F:15, J:3	274-350, 443-513	76, 293
gbs1145	surface exclusion protein Sec10	4-22,29-41,45-51,53-61,70-76,85-92,99-104,111-122,134-140,142-154,163-174,224-232,255-265,273-279,283-297,330-335,337-348,356-367,373-385,391-396,421-431,442-455,475-485,493-505,526-538,544-561,587-599,605-620,622-651,662-670,675-681,687-692,697-712,714-735	C:2	252-262	77, 294
gbs1158	Similar to oxidoreductase	4-12,15-35,40-46,50-59,67-94,110-128,143-169,182-188,207-215,218-228,238-250	K:2	74-90	78, 295
gbs1165	cysteine desulphurase (iscS-1)	9-18,42-58,78-85,88-95,97-106,115-122,128-134,140-145,154-181,186-202,204-223,261-267,269-278,284-	F:5	12-29	79, 296

S. agalactiae antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immunogenic region (aa)	Seq. ID (DNA, Prot.)
		293,300-336,358-368			
gbs1195	staphylokinase and streptokinase	7-34,46-53,62-72,82-88,100-105,111-117,132-137,144-160,166-180,183-189,209-221,231-236,246-253,268-282,286-293,323-336,364-372,378-392,422-433	B:3, C:2, D:2, G:3, H:8	388-405	80, 297
gbs1209	ATP-dependent DNA helicase PcrA	21-27,34-50,72-77,80-95,164-177,192-198,202-220,226-236,239-247,270-279,285-292,315-320,327-334,348-355,364-371,388-397,453-476,488-497,534-545,556-576,582-588,601-607,609-616,642-662,674-681,687-697,709-715,721-727,741-755	C:3, I:5	621-739	81, 298
gbs1214	conserved hypothetical protein	4-14,16-77,79-109	B:2	25-99	82, 299
gbs1242	CpsG, beta-1,4-galactosyltransferase	4-9,17-23,30-37,44-55,65-72,77-93,102-121,123-132,146-153	L:24	17-29	83, 300
gbs1260	ABC transporter, ATP-binding protein	4-18,25-41,52-60,83-92,104-112,117-123,149-155,159-167,170-192,201-210,220-227,245-250	I:17	124-137	84, 301
gbs1270	gbs1270 hyaluronate lyase	8-25,50-55,89-95,138-143,148-153,159-169,173-179,223-238,262-268,288-295,297-308,325-335,403-409,411-417,432-446,463-475,492-501,524-530,542-548,561-574,576-593,604-609,612-622,637-654,665-672,678-685,720-725,731-739,762-767,777-783,820-838,851-865,901-908,913-920,958-970,1000-1006,1009-1015,1020-1026,1043-1052,1055-1061	C:19, D:5, L:19	1-128, 252-341, 771-793, 1043-1058	85, 302
gbs1305	hypothetical protein	16-26,33-46	I:2	64-76	86, 303
gbs1306	Laminin binding protein	4-27,69-77,79-101,117-123,126-142,155-161,171-186,200-206,213-231,233-244,267-273,313-329,335-344,347-370,374-379,399-408,422-443,445-453,461-468,476-482,518-534,544-553,556-567,578-595,601-620,626-636,646-658,666-681,715-721,762-768,778-785,789-803,809-819	A:6, B:7, C:17, D:72, E:8, F:91, G:2, H:4, I:26, J:3, K:14	22-108, 153-318, 391-527, 638-757	87, 304
gbs1307	Lmb, laminin-binding surface protein	6-21,32-43,62-92,104-123,135-141,145-152,199-216,218-226,237-247,260-269,274-283,297-303	A:2, D:3	1-72, 127-211	88, 305
gbs1308	C5a peptidase, authentic frameshift	6-26,50-56,83-89,108-114,123-131,172-181,194-200,221-238,241-247,251-259,263-271,284-292,304-319,321-335,353-358,384-391,408-417,424-430,442-448,459-466,487-500,514-528,541-556,572-578,595-601,605-613,620-631,635-648,660-670,673-679,686-693,702-	B:4, C:15, D:70, E:18, F:26, G:5, H:4, J:2, K:40	1-213, 269-592, 992-1120	89, 306

S. agalactiae antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immunogenic region (aa)	Seq. ID (DNA, Prot.)
		708,716-725,730-735,749-755,770-777,805-811,831-837,843-851,854-860,863-869,895-901,904-914,922-929,933-938,947-952,956-963,1000-1005,1008-1014,1021-1030,1097-1103,1120-1130,1132-1140			
gbs1309	hypothetical protein	9-16,33-39,47-59,65-79,81-95,103-108,115-123,138-148,163-171,176-185,191-196,205-211,213-221,224-256,261-276,294-302,357-363,384-390	E:2, F:4, H:2, J:2	95-111, 161-189	90, 307
gbs1311	transposase, C-terminal part	21-27,35-45,70-76,92-105,129-143,145-155,161-166,170-191,204-211,214-231,234-246,249-255,259-275	F:3	1-18	91, 308
gbs1321	hypothetical protein	21-35,45-53,56-64,69-97	F:7	1-16	92, 309
gbs1352	putative helicase and methylase	25-33,41-47,61-68,86-101,106-114,116-129,134-142,144-156,163-176,181-190,228-251,255-261,276-292,295-305,334-357,368-380,395-410,424-429,454-460,469-482,510-516,518-527,531-546,558-570,579-606,628-636,638-645,651-656,668-674,691-698,717-734,742-754,765-770,792-797,827-835,847-859,874-881,903-909,926-933,942-961,964-977,989-1004,1010-1028,1031-1047,1057-1075,1081-1095,1108-1117,1138-1144,1182-1189,1193-1206,1220-1229,1239-1246,1257-1267,1271-1279,1284-1301,1312-1320,1329-1335,1341-1347,1358-1371,1399-1404,1417-1426,1458-1463,1468-1476,1478-1485,1493-1506,1535-1541,1559-1574,1583-1590,1595-1601,1603-1611,1622-1628,1634-1644,1671-1685,1689-1696,1715-1720,1734-1746,1766-1775,1801-1806,1838-1844,1858-1871,1910-1917,1948-1955,1960-1974,2000-2015,2019-2036,2041-2063	E:3, H:2, M:4	748-847, 1381-1391	93, 310
gbs1356	Putative peptidoglycan linked protein (LPXTG motif) - Agglutinin receptor	5-12,18-24,27-53,56-63,96-113,119-124,131-136,157-163,203-209,215-223,233-246,264-273,311-316,380-389,393-399,425-433,445-450,457-462,464-470,475-482,507-513,527-535,542-548,550-565,591-602,607-613,627-642,644-664,673-712,714-732,739-764,769-782,812-818,826-838,848-854,860-871,892-906,930-938,940-954,957-973,990-998,1002-1021,1024-1033,1037-1042,1050-1060,1077-1083,1085-1092,1100-1129,1144-1161,1169-1175,1178-1189,1192-1198,1201-1207,1211-1221,1229-1239,1250-1270,1278-1292,1294-1300,1314-1335,1344-1352,1360-1374,1394-1405,1407-1414,1416-1424,1432-1452,1456-1462,1474-1497,1500-1510,1516-	C:5, D:62, I:22	187-273, 306-441	94, 311

S. agalactiae antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immunogenic region (aa)	Seq. ID (DNA, Prot.)
		1522,1534-1542,1550-1559,1584-1603,1608-1627			
gbs1376	similar to ATP-dependent Clp proteinase (ATP-binding subunit), ClpL	70-80,90-97,118-125,128-140,142-148,154-162,189-202,214-222,224-232,254-260,275-313,317-332,355-360,392-398,425-432,448-456,464-470,476-482,491-505,521-528,533-546,560-567,592-597,605-614,618-626,637-644,646-653,660-666,677-691	K:4	207-227	95, 312
gbs1377	similar to homocysteine S-methyltransferase	5-19,26-34,37-55,57-66,69-83,86-102,115-134,138-143,154-172,178-195,209-246,251-257,290-302,306-311	M:2	256-266	96, 313
gbs1386	hydroxy-3-methylglutaryl-coenzyme A synthase	10-20,22-28,35-57,72-79,87-103,108-128,130-144,158-171,190-198,225-242,274-291,301-315,317-324,374-385	G:2	353-365	97, 314
gbs1390	hypothetical protein	4-9,17-30,34-54,59-66,73-94,118-130,135-150,158-171,189-198,219-239,269-275,283-301	E:3, K:4	89-106, 176-193	98, 315
gbs1391	hypothetical protein	14-20,22-74,77-86,89-99,104-109,126-135,154-165,181-195,197-212,216-224,264-275	E:3	107-118	99, 316
gbs1403	similar to 5'-nucleotidase, putative peptidoglycan bound protein (LPXTN)	4-18,21-38,63-72,101-109,156-162,165-179,183-192,195-210,212-218,230-239,241-256,278-290,299-311,313-322,332-341,348-366,386-401,420-426,435-450,455-460,468-479,491-498,510-518,532-538,545-552,557-563,567-573,586-595,599-609,620-626,628-636,652-657,665-681	A:3, C:12, D:4, J:2	1-198	100, 317
gbs1408	Similar to ABC transporter (ATP-binding protein)	4-10,16-38,51-68,73-79,94-115,120-125,132-178,201-208,216-223,238-266,269-295,297-304,337-342,347-356,374-401,403-422,440-447,478-504,510-516,519-530,537-544	D:2, K:4	191-206	101, 318
gbs1420	similar to cell wall proteins, putative peptidoglycan linked protein (LPXTG motif)	12-40,42-48,66-71,77-86,95-102,113-120,129-137,141-148,155-174,208-214,218-225,234-240,256-267,275-283,300-306,313-321,343-350,359-367,370-383,398-405,432-439,443-461,492-508,516-526,528-535	C:3, D:4	370-478	102, 319
gbs1429	hypothetical protein	6-14,20-37,56-62,90-95,97-113,118-125,140-145,161-170,183-202,237-244,275-284,286-305,309-316,333-359,373-401,405-412	B:2, C:2	176-187	103, 320
gbs1442	hypothetical thiamine biosynthesis protein, ThiI	33-44,50-55,59-80,86-101,129-139,147-153,157-163,171-176,189-201,203-224,239-245,257-262,281-287,290-297,304-320,322-331,334-350,372-390,396-401	L:28	71-88, 353-372	104, 321

S. agalactiae antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immunogenic region (aa)	Seq. ID (DNA, Prot.)
gbs1452	rplT 50S ribosomal protein L20	5-11,15-24,26-33,40-47,75-88,95-103,105-112	E:2	17-30	105, 322
gbs1464	ferrichrome ABC transporter (permease)	5-11,16-39,46-54,62-82,100-107,111-124,126-150,154-165,167-183,204-238,245-295,301-313,316-335	F:4	8-16	106, 323
gbs1470	conserved hypothetical protein	4-19,34-48,69-74,79-107,115-127,129-135,143-153,160-169,171-182	I:4	142-153	107, 324
gbs1528	conserved hypothetical protein	4-30,65-74,82-106,110-120,124-132,135-140,146-175,179-184,190-196,217-223,228-233,250-267,275-292,303-315,322-332	I:7	174-186	108, 325
gbs1529	Putative peptidoglycan bound protein (LPXTG motif)	9-16,29-41,47-57,68-84,87-109,113-119,162-180,186-193,195-201,203-208,218-230,234-243,265-271,281-292,305-312,323-332,341-347,349-363,368-374,383-390,396-410,434-440,446-452,455-464,466-473,515-522,529-542,565-570,589-600,602-613,618-623,637-644,1019-1027,1238-1244,1258-1264,1268-1276,1281-1292,1296-1302	C:2	883-936	109, 326
gbs1531	UvrB excinuclease ABC chain B	10-17,23-32,39-44,54-72,75-81,88-111,138-154,160-167,178-185,201-210,236-252,327-334,336-342,366-376,388-400,410-430,472-482,493-526,552-558,586-592,598-603,612-621,630-635,641-660	M:2	384-393	110, 327
gbs1533	glutamine ABC transporter, glutamine-binding protein	4-22,24-39,50-59,73-84,100-105,111-117,130-138,155-161,173-178,182-189,205-215,266-284,308-313,321-328,330-337,346-363,368-374,388-395,397-405,426-434,453-459,482-492,501-507,509-515,518-523,527-544,559-590,598-612,614-629,646-659,663-684,686-694,698-721	I:4	445-461	111, 328
gbs1536	hypothetical protein	14-22,27-33	E:10	3-17	112, 329
gbs1542	oxidoreductase, aldo/keto reductase family	29-41,66-73,81-87,90-108,140-146,150-159,165-184,186-196,216-226,230-238,247-253,261-269	I:13	126-140	113, 330
gbs1547	small protein, SmpB	5-12,16-25,27-33,36-45,60-68,83-88,103-126	L:11	86-101	114, 331
gbs1565	hypothetical protein	14-23,36-47,56-66,84-89,94-105,111-127,140-153,160-174,176-183,189-203,219-225,231-237,250-257	F:2, J:2, K:8, L:21	194-227	115, 332
gbs1586	peptidyl-prolyl cis-trans isomerase, cyclophilin-type	4-25,54-60,64-71,73-82,89-106,117-124,157-169,183-188,199-210,221-232,236-244,255-264	E:3	58-98	116, 333

S. agalactiae antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immunogenic region (aa)	Seq. ID (DNA, Prot.)
gbs1591	5-methylthioadenosine nucleosidase/S-adenosylhomocysteine nucleosidase (pfs)	13-19,26-36,41-53,55-71,77-84,86-108,114-135,157-172,177-183,187-194,208-213,218-226	I:18, L:2	110-125, 156-170	117, 334
gbs1632	similar to branched-chain amino acid ABC transporter, amino acid-binding protein	5-24,63-69,77-85,94-112,120-137,140-146,152-159,166-172,179-187,193-199,206-212,222-228,234-240,244-252,257-264,270-289,298-309,316-328,337-348,363-375	B:2, E:4, I:3	1-56, 340-352	118, 335
gbs1638	amino acid permease	18-39,42-71,78-120,124-144,152-173,179-189,199-209,213-222,228-258,269-304,329-361,364-372,374-389,396-441	E:8, G:9, H:9	313-327	119, 336
gbs1662	conserved hypothetical protein	19-25,91-98,108-120,156-162,168-174,191-204,211-216,232-266,272-278,286-308,316-321,327-333,344-355,358-364,384-391,395-428,464-476,487-495,497-511,544-561,563-573,575-582,588-594	E:3, H:2	10-25, 322-338	120, 337
gbs1666	SWI/SNF family helicase	14-26,32-49,51-57,59-72,80-91,102-112,119-125,147-161,164-173,175-183,188-213,217-222,246-254,260-276,282-303,308-318,321-328,333-350,352-359,371-378,392-401,407-414,416-443,448-463,471-484,490-497,501-514,519-527,539-551,557-570,578-590,592-598,600-610,618-629,633-647,654-667,676-689,702-709,718-726,728-737,741-760,764-780,786-795,808-826,836-842,845-852,865-874,881-887,931-945,949-957,968-974,979-986,1003-1009,1023-1029	F:4	90-103	121, 338
gbs1673	conserved hypothetical protein	11-16,37-56,60-66,69-77,80-88,93-106,117-139,166-171	E:2	72-90	122, 339
gbs1695	dihydroxyacetone kinase family protein	59-84,123-133,145-150,161-167,178-189	I:8	115-128	123, 340
gbs1754	excinuclease ABC, A subunit (uvrA)	15-33,39-46,52-64,74-87,108-124,127-144,150-156,173-179,184-194,201-208,219-236,243-269,272-295,302-309,343-349,356-361,370-379,405-411,414-423,430-451,457-464,466-475,477-483,496-502,507-522,541-548,557-563,571-577,579-585,590-605,626-642,650-662,671-691,704-710,751-769,775-781,786-791,794-829,851-858,868-878,884-904,913-919,931-939	I:2	132-142	124, 341
gbs1760	Similar to A/G-specific adenine	33-58,64-71,74-80,83-88,96-120,122-139,146-157,167-177,207-213,220-225,236-242,264-279,300-305,326-	K:8	97-115, 199-211	125, 342

S. agalactiae antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immunogenic region (aa)	Seq. ID (DNA, Prot.)
	glycosylase	336,340-347,350-360			
gbs1777	glycerol uptake facilitator protein, putative	4-26,43-57,70-99,102-117,121-133,142-148,151-168,170-183,192-220,235-249,258-279	E:4	30-41	126, 343
gbs1783	polyprenyl synthetase family protein	34-42,48-58,70-94,110-130,154-160,164-172,178-183,195-203,211-222,229-250,256-261,274-284,286-292,312-323	I:3	222-233	127, 344
gbs1784	ABC transporter, ATP-binding protein CydC	4-9,15-36,38-45,49-74,78-88,100-112,136-191,211-220,226-233,239-246,254-274,287-307,316-322,342-353,356-366,373-378,384-393,405-431,449-457,459-468,487-511,515-524,529-541,544-552,562-568,571-576	C:2, D:2	208-280	128, 345
gbs1790	hypothetical protein	10-27,31-37,39-54,71-108,124-143	A:23, C:6	2-107	129, 346
gbs1805	Similar to secreted unknown protein	16-27,38-57,64-70,90-102,104-113,116-137,160-166	A:197, C:2	1-80	130, 347
gbs1816	HD domain protein	13-21,31-36,56-67,127-136,153-171,173-180,184-200,214-222,225-231,239-263,267-273	F:8	135-159	131, 348
gbs1821	Similar to 23S ribosomal RNA methyltransferase	12-27,31-51,68-74,77-87,94-101,108-114,117-123,127-134,138-168,173-196,201-207,212-217,227-237,247-257,264-280	K:5	205-223	132, 349
gbs1823	triad family protein	17-22,25-54,70-76,92-100	G:6, H:3	98-110	133, 350
gbs1834	two-component sensor histidine kinase	7-29,40-50,60-67,87-96,105-111,119-164,172-199,206-212,220-227,237-259,272-279,282-293,295-309,313-319,321-328,345-363,376-386	E:3, F:6	159-176	134, 351
gbs1842	transcriptional antiterminator, BglG family	4-19,24-30,36-43,50-68,71-89,93-106,141-152,154-172,179-197,199-215,229-239,246-252,255-263,281-298,319-325,329-356,358-368,374-390,397-409,420-429,432-444,450-456,459-475,483-494,496-502,520-528,532-556	I:19	362-377	135, 352
gbs1850	hypothetical transaldolase	18-25,40-62,77-85,91-97,105-116,123-133,139-184,189-197	G:2	122-140	136, 353
gbs1869	phosphoglycerate kinase	4-49,52-58,62-70,79-105,109-133,142-150,163-168,206-214,220-228,233-240,243-254,274-281,303-311,327-338,357-373,378-396,403-413,420-436,441-453,461-467,475-481,484-498,506-512,514-521,523-529,562-579,589-595,598-603,615-648,714-722,728-742,749-758,777-792,795-807	L:9	643-658	137, 354
gbs1875	alkyl hydroperoxide	8-27,37-48,51-56,72-79,87-106,120-138,140-147,167-	F:3	456-470	138, 355

S. agalactiae antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immunogenic region (aa)	Seq. ID (DNA, Prot.)
	reductase (large subunit) and NADH dehydrogenase	176,187-197,205-216,222-229,234-239,243-249,277-288,292-315,334-343,347-353,363-391,398-404,430-447,461-467,478-492,498-507			
gbs1879	endopeptidase O (pepO)	5-12,18-24,59-69,80-93,95-109,119-125,130-137,139-147,158-163,168-176,182-202,206-215,222-239,241-249,267-277,291-298,311-318,321-327,338-344,348-355,373-386,393-406,411-417,434-443,446-465,473-484,514-521,532-553,584-594	I:26	221-237	139, 356
gbs1893	2-keto-3-deoxygluconate kinase	4-14,27-34,50-58,63-72,79-106,109-114,121-142,146-154,161-167,169-175,178-201,223-238,249-254,259-264,278-292,294-312,319-330	F:8, K:9	167-191	140, 357
gbs1899	N-acetylmuramoyl-L-alanine amidase, family 4 protein	7-28,36-42,50-61,63-80,122-152,161-174,176-191	B:2, C:2, E:3	140-190	141, 358
gbs1907	citrate carrier protein, CCS family	20-57,59-65,70-78,86-102,119-133,142-161,163-173,177-188,192-202,204-220,222-236,240-253,279-319,326-331,337-383,390-399,406-412,420-427,431-438	I:2	381-395	142, 359
gbs1924	similar to pneumococcal histidine triad protein B precursor (C-terminal part)	13-18,28-34,37-43,50-59,75-81,83-97,105-121,139-147,200-206,209-227,231-247,260-271,318-327,366-381,388-394,399-406	K:3	182-201	143, 360
gbs1925	similar to pneumococcal histidine triad protein B precursor (N-terminal part)	6-29,37-43,51-56,70-77,82-102,110-119,127-143,178-190,201-209,216-243,261-269,281-292,305-313,327-339,341-354,356-373,391-397,423-429,438-445,450-478	A:2, B:5, C:12, D:57	21-314	144, 361
gbs1962	conserved hypothetical protein	4-12,15-21,32-41,59-76,80-89,96-104	E:3	90-103	145, 362
gbs2008	similar to C5A peptidase, putative peptidoglycan linked protein (LPXTG motif)	9-28,30-41,44-54,69-74,77-82,90-97,104-123,125-135,149-155,164-173,177-184,217-226,230-235,238-244,258-272,282-297,300-305,309-315,317-322,327-336,348-362,368-374,380-387,400-411,414-424,451-458,460-466,483-494,497-503,506-511,521-528,540-553,569-587,598-606,628-642,661-681,688-700,718-733,740-749,752-764,769-783,823-834,848-854,862-872,878-884,886-898,915-920,938-951,954-961,963-972,982-	A:253, B:2, C:3, D:6, H:2	3-82, 509-576	146, 363

S. agalactiae antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immunogenic region (aa)	Seq. ID (DNA, Prot.)
		989,996-1003,1010-1016,1021-1032,1038-1044,1047-1057,1060-1070,1079-1088,1094-1102,1117-1127,1129-1135,1142-1153,1158-1204,1212-1229,1234-1263,1269-1277,1308-1313,1327-1338,1344-1376,1400-1415,1436-1443,1448-1458,1497-1504,1511-1522,1544-1566			
gbs2018	putative peptidoglycan linked protein (LPXTG motif)	8-36,40-64,71-79,88-94,102-109,118-127,138-148,151-159,163-174,192-198,200-206,220-233,268-273,290-301,304-309,316-323,331-349,378-391,414-420,427-437,455-475,494-510,541-547,549-555,616-640	A:132, B:6, C:13, D63, E:15, H:2, J:9, K:13	1-60, 55-139, 212-308, 386-458, 458-624	147, 364
gbs2029	hypothetical protein	16-31,35-42,70-77,91-101,120-130,132-140,143-153,185-190,195-202,215-222,228-238,241-251,257-264,268-277,288-302,312-324,326-333,341-348,364-382,415-429,438-454,458-466,491-499,501-521	G:8	273-281	148, 365
gbs2042	hypothetical protein	8-14,32-57,74-149,155-177,179-212,221-266,271-296,304-324,329-346,349-359,368-401,413-419,426-454,465-478,493-510	L:11	466-490	149, 366
gbs2054	DNA mismatch repair protein HexA	22-28,33-51,64-89,96-119,126-132,138-146,152-159,161-169,172-179,193-198,205-211,221-231,235-254,273-280,297-303,312-320,328-346,351-373,378-384,391-398,448-454,460-468,470-481,516-558,574-593,597-602,613-623,626-646,649-656,668-673,675-683,696-708,715-722,724-739,745-751,759-777,780-804,816-822	E:8	102-113	150, 367
gbs2058	hypothetical protein	12-28,41-91,98-107,112-120,125-131,151-193,215-221,240-250,263-280	L:3	128-138	151, 368
gbs2060	aspartyl-tRNA synthetase (aspS)	16-24,32-38,46-62,68-81,90-105,127-133,144-150,160-166,178-184,186-202,210-219,232-240,252-258,264-273,293-324,337-344,349-357,360-369,385-398,410-416,419-427,441-449,458-476,508-515,523-539,544-549,562-569,571-579	I:3, L:12	96-109, 127-139	152, 369
gbs2075	hypothetical protein	19-25,28-34,56-61,85-97,110-116	M:2	39-53	153, 370
gbs2106	protein of unknown function/lipoprotein, putative	4-37,41-50,62-72,91-97,99-109,114-125,136-141,149-158,160-166,201-215	A:5, B:6, C:4, D:14, E:11, I:8, K:23	27-225	154, 371
gbs2118	similar to inosine monophosphate dehydrogenase	15-31,44-51,96-105,122-130,149-157,162-168,178-183,185-192,198-204,206-213,221-234,239-245,248-255,257-266,289-335,349-357,415-422,425-441,448-454,462-468	K:17	463-481	155, 372

S. agalactiae antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immunogenic region (aa)	Seq. ID (DNA, Prot.)
gbs2131	ABC transporter, permease protein, putative	5-31,39-55,63-72,76-99,106-155,160-177,179-199,207-217,223-240,245-255,261-267,294-316,321-343,354-378,382-452,477-488,529-536,555-569,584-591,593-612,620-627,632-640,647-654,671-680,698-704,723-730,732-750,769-775,781-788,822-852	I:2	505-525	156, 373
ARF0112	Hypothetical protein	none	F:6	3-18	157, 374
ARF0147	Hypothetical protein	4-14	E:3, I:3	12-24	158, 375
ARF0532	Hypothetical protein	4-11,22-30	F:10	12-25	159, 376
ARF0534	Hypothetical protein	5-12	E:2, G:2	4-18	160, 377
ARF0557	Hypothetical protein	4-28	E:2, G:6, H:4	7-14	161, 378
ARF0862	Hypothetical protein	6-16	G:7, H:4	8-16	162, 379
ARF0891	Hypothetical protein	4-15,18-33	K:6	24-36	163, 380
ARF0895	Hypothetical protein	4-10,16-21	I :21	20-31	164, 381
ARF0943	Hypothetical protein	none	C:2, K:9	6-19	165, 382
ARF0973	Hypothetical protein	11-18	D:2, G:3, H:8, I:2, K:2	3-10	166, 383
ARF0999	Hypothetical protein	13-24	B:4, K:3	3-15	167, 384
ARF1010	Hypothetical protein	15-27	K:2	7-16	168, 385
ARF1230	Hypothetical protein	11-16	K:11	1-15	169, 386
ARF1503	Hypothetical protein	4-16	E:13	9-21	170, 387
ARF1556	Hypothetical protein	4-24,40-48,54-67	F:2	22-39	171, 388
ARF1585	Hypothetical protein	6-30,34-55,62-68,78-106	I:5, J:4	68-74	172, 389
ARF1588	Hypothetical protein	none	I:2	3-14	173, 390
ARF1735	Hypothetical protein	9-19	I:13	6-21	174, 391
ARF1809	Hypothetical protein	4-17	H:2, L:17	1-9	175, 392
ARF1826	Hypothetical protein	5-30	I:6	1-8	176, 393
ARF1882	Hypothetical protein	4-16,23-46,51-56	K:23	45-55	177, 394
ARF1996	Hypothetical protein	none	F:3	7-16	178, 395
CRF0123	Hypothetical protein	none	F:32	2-14	179, 396
CRF0180	Hypothetical protein	4-36,43-65	E:6, G:6, H:12	50-62	180, 397
CRF0208	Hypothetical protein	10-30	I:2	14-21	181, 398
CRF0258	Hypothetical protein	9-17	I:2	1-10	182, 399
CRF0285	Hypothetical protein	4-12	F:2	3-16	183, 400
CRF0311	Hypothetical protein	4-15	H:4	5-23	184, 401
CRF0446	Hypothetical protein	none	L:20	10-21	185, 402

S. agalactiae antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immunogenic region (aa)	Seq. ID (DNA, Prot.)
CRF0455	Hypothetical protein	none	F:5	6-16	186, 403
CRF0491	Hypothetical protein	4-29,31-38	G:4	2-14	187, 404
CRF0520	Hypothetical protein	4-35	H:4	33-42	188, 405
CRF0530	Hypothetical protein	none	G:13, H:8, K:3	2-17	189, 406
CRF0570	Hypothetical protein	9-18,30-35	I:2	15-33	190, 407
CRF0649	Hypothetical protein	4-9	G:8, H:6	6-12	191, 408
CRF0853	Hypothetical protein	none	I:6	3-17	192, 409
CRF0955	Hypothetical protein	12-21,37-44,52-61,72-80	E:7, L:44	38-48	193, 410
CRF0983.1	Hypothetical protein	4-10,29-44,54-61,69-78	K:59	13-27	194, 411
CRF0983.2	Hypothetical protein	13-23,36-53	L:33	2-15	195, 412
CRF1083	Hypothetical protein	4-25,28-46,56-72,81-99,120-132,134-142,154-160	F:18	129-141	196, 413
CRF1095	Hypothetical protein	4-15,24-33,35-41,64-86	L:15	21-33	197, 414
CRF1212.1	Hypothetical protein	9-15	I:5	4-13	198, 415
CRF1212.2	Hypothetical protein	4-11,13-19,34-48	L:30	15-32	199, 416
CRF1290	Hypothetical protein	4-21	I:7	11-31	200, 417
CRF1383	Hypothetical protein	23-57	K:13	38-50	201, 418
CRF1416	Hypothetical protein	4-32	E:16, J:7	3-13	202, 419
CRF1500	Hypothetical protein	4-10,13-25,32-42,56-68,72-84	E:16	26-38	203, 420
CRF1513	Hypothetical protein	4-20,31-48,52-58,65-71,80-93,99-108,114-123	I:2	37-49	204, 421
CRF1518	Hypothetical protein	6-12,14-20	F:28	3-25	205, 422
CRF1663	Hypothetical protein	14-25,27-38	F:10	5-14	206, 423
CRF1667	Hypothetical protein	4-41,57-105,109-118,123-136,144-152	G:4	86-99	207, 424
CRF1832	Hypothetical protein	None	E:5, L:8	6-19	208, 425
CRF1866	Hypothetical protein	none	G:3, H: 18	2-19	209, 426
CRF1892	Hypothetical protein	14-47	L:11	1-14	210, 427
CRF1942	Hypothetical protein	4-21,29-44	F:14	2-18	211, 428
CRF1992	Hypothetical protein	23-29	K:10	10-28	212, 429
CRF2047	Hypothetical protein	6-16,22-36	K:9	11-22	213, 430
CRF2050	Hypothetical protein	4-19,30-44	I:2	18-27	214, 431
CRF2096	Hypothetical protein	5-15,37-45,58-65	G:2	38-47	215, 432
CRF2113	Hypothetical protein	4-15,23-34	I:5	4-15	216, 433
NRF1311	transposase, C-terminal part	30-36,44-54,79-85,101-114,138-152,154-164,170-175,179-200,213-220,223-240,243-255,258-264,268-284	F:3	10-28	217, 434

Table 1B: Immunogenic proteins identified by amino acid sequence identity with peptides identified by bacterial surface display.

S. agalactiae antigenic protein (new)	Identical region	Peptide sequence	Peptide name	Protein identified by BSD	Immunogenic region (aa)	Sequence ID (DNA, protein)
gbs0384	210 – 226	MEYKGNFSQKTINRFKS	gbs0995.1	gbs0995	210 – 226	435, 449
	738 –753	QTQRSGKINTDFMRQL	gbs0995.2	gbs0995	738 -753	
gbs0393	326 – 344	VKTIGYGKLTGKVNHHYVA	gbs0986.2	gbs0986	326 – 344	436, 450
	326 – 348	VKTIGYGKLTGKVNHHYVANKDG	gbs1143.1	gbs1143	327 - 349	
	338 – 354	VNHHYVANKDGSVTAFV	gbs0986.3	gbs0986	338 – 354	
	371 – 392	AAVNQNIVFRVLTKDGRPIFEK	gbs1143.2	gbs1143	372 - 393	
	801 – 809	TVIKKGTNL	gbs0986.4	gbs0986	801 – 809	
	877 – 901	VTHTTEKSKPVEPQKATPKAPAKGL	gbs0986.5	gbs0986	877 - 901	
gbs0396	893 - 906	RQELLTPTQLSKLQ	gbs0983.1	gbs0983	893 - 906	437, 451
gbs0407	51 - 69	VRYDKLEALVAYHGAKSAS	gbs0972.1	gbs0972	51 - 69	438, 452
gbs0408	110 - 125	HQPNRIYLTDKLVPYI	gbs0971.1	gbs0971	110 - 125	439, 453
gbs0410	291 - 305	QSIKQHDKEKLRTVL	gbs0969.1	gbs0969	291 - 305	440, 454
gbs0714	210 - 226	MEYKGNFSQKTINRFKS	gbs0995.1	gbs0995	210 - 226	441, 455
	738 -753	QTQRSGKINTDFMRQL	gbs0995.2	gbs0995	738 -753	
gbs0723	326 – 344	VKTIGYGKLTGKVNHHYVA	gbs0986.2	gbs0986	326 – 344	442, 456
	326 – 348	VKTIGYGKLTGKVNHHYVANKDG	gbs1143.1	gbs1143	327 - 349	
	338 – 354	VNHHYVANKDGSVTAFV	gbs0986.3	gbs0986	338 – 354	
	371 – 392	AAVNQNIVFRVLTKDGRPIFEK	gbs1143.2	gbs1143	372 - 393	
	801 – 809	TVIKKGTNL	gbs0986.4	gbs0986	801 – 809	
	877 – 901	VTHTTEKSKPVEPQKATPKAPAKGL	gbs0986.5	gbs0986	877 - 901	
gbs0726	893 - 906	RQELLTPTQLSKLQ	gbs0983.1	gbs0983	893 - 906	443, 457
gbs0737	51 - 69	VRYDKLEALVAYHGAKSAS	gbs0972.1	gbs0972	51 - 69	444, 458
gbs0738	110 - 125	HQPNRIYLTDKLVPYI	gbs0971.1	gbs0971	110 - 125	445, 459
gbs0740	291 - 305	QSIKQHDKEKLRTVL	gbs0969.1	gbs0969	291 - 305	446, 460
gbs0897	32 - 44	EGDVLLEIMSDKT	gbs0898.1	gbs0898	32 - 44	447, 461
gbs0966	399 -410	PGLTVEEKFVTF	gbs0144.1	gbs0144	420 - 431	448, 462

Table 2. Epitope serology with human sera

Peptides	positivity	aa from	aa to	Seq ID
gbs0012.1	++	120	143	218
gbs0012.2	+	138	161	218
gbs0012.3	+	156	179	218
gbs0016.2	+++	110	129	219
gbs0016.3	+	168	184	219
gbs0048.1	+	74	90	222
gbs0053.1	+++	759	773	223
gbs0061.1	+++	237	260	224
gbs0084.1	+	265	284	225
gbs0107.1	++	65	74	226
gbs0108.1	++	41	50	227
gbs0123.1	+	163	174	229
gbs0127.1	++	26	37	230
gbs0183.1	+	174	189	232
gbs0235.1	++	240	256	234
gbs0260.1	+	285	297	236
gbs0286.1	+	238	247	238
gbs0288.1	+	491	519	239
gbs0437.1	++	114	140	243
gbs0539.1	+	267	284	250
gbs0579.1	+	439	453	252
gbs0580.1	++	162	178	253
gbs0628.1	++	347	364	254
gbs0632.1	+++	699	715	255
gbs0634.1	+	60	71	256
gbs0667.1	++	244	257	257
gbs0672.1	+	44	63	258
gbs0672.2	+	57	76	258
gbs0785.1	+	185	196	260
gbs0851.1	+	119	129	263
gbs0896.1	++	182	195	266
gbs0898.1	++	32	44	267
gbs0898.2	+	424	442	267
gbs0904.1	+	247	256	268
gbs0918.1	++	678	694	269
gbs0918.2	+	785	805	269
gbs0918.4	+	55	77	269
gbs0918.5	+++	72	94	269
gbs0995.1	+	210	226	281
gbs1087.3	+	37	59	289
gbs1165.1	+	13	29	296
gbs1816.1	+	136	159	348
gbs1821.1	+	205	222	349
gbs1823.1	+	99	110	350
gbs1834.1	+	160	176	351
gbs1875.1	+	457	470	355
gbs1879.1	+	221	237	356
gbs1893.1	+	167	190	357
gbs1925.1	+	96	120	361
gbs2018.3	+++	399	417	364
gbs2018.4	+++	503	519	364
gbs2018.5	+++	544	563	364
gbs2106.2	+	46	68	371
gbs2106.7	+	159	183	371
gbs2106.8	+	184	198	371
gbs2118.1	++	463	481	372

Table 3: Gene distribution in *S. agalactiae* strains.

ORF	Common name	Gene distribution (present of 50)	Amino acid substitutions (in serotype IA strain)*	Seq. ID (DNA, Prot.)
gbs0012	weakly similar to beta-lactamase	44/44	n.d.	1, 218
gbs0016	glucan-binding protein B (S.mutans)	40/44	0/224	2, 219
gbs0024	Phosphoribosylformylglycinamidine	46/46	10/228	3, 220
gbs0031	surface immunogenic protein	46/46	1/225	4, 221
gbs0048	Unknown	30/46	0/61	5, 222
gbs0053	aldehyde-alcohol dehydrogenase (adhE)	45/45	0/224	6, 223
gbs0061	rplB ribosomal protein L2	46/46	0/218	7, 224
gbs0084	DNA-directed RNA polymerase, alpha subunit (rpoA)	45/45	0/207	8, 225
gbs0107	conserved hypothetical protein	46/46	0/235	9, 226
gbs0108	deoxyuridine 5`-triphosphate nucleotidohydrolase	44/44	0/125	10, 227
gbs0113	ribose ABC transporter	44/45	0/227	11, 228
gbs0123	similar to argininosuccinate synthase	44/44	0/184	12, 229
gbs0127	rpmV 50S ribosomal protein L28	46/46	0/40	13, 230
gbs0144	oligopeptide ABC transporter, substrate-binding	45/45	0/282	14, 231
gbs0183	membrane protein, putative	44/44	0/223	15, 232
gbs0184	oligopeptide ABC transporter, oligopeptide-binding	46/46	1/203	16, 233
gbs0235	glycine betaine/carnitine/choline ABC transporter	46/46	0/219	17, 234
gbs0255	conserved hypothetical protein	46/46	0/180	18, 235
gbs0260	glycyl-tRNA synthetase (beta subunit	46/46	0/209	19, 236
gbs0268	transketolase (tkt)	46/46	0/208	20, 237
gbs0286	NH3-dependent NAD+ synthetase	45/45	0/191	21, 238
gbs0288	similar to penicillin-binding protein 1A	45/45	0/212	22, 239
gbs0343	seryl-tRNA synthetase (serS)	46/46	0/228	23, 240
gbs0428	similar to fibrinogen binding protein, putative peptidoglycan linked protein (LPXTG motif)	45/46	1/126	25, 242
gbs0437	glucose-6-phosphate isomerase (pgi)	45/45	0/232	26, 243
gbs0460	decarboxylase	46/46	1/81	27, 244

ORF	Common name	Gene distribution (present of 50)	Amino acid substitutions (in serotype IA strain)*	Seq. ID (DNA, Prot.)
gbs0465	oxydoreductase	46/46	0/126	28, 245
gbs0489	acetyltransferase, GNAT family	45/45	3/144	30, 247
gbs0492	gbs0492 valyl-tRNA synthetase	44/44	3/125	31, 248
gbs0538	amino acid ABC transporter (ATP-binding protein)	46/46	0/214	32, 249
gbs0539	similar to phosphomannomutase	46/46	0/244	33, 250
gbs0555	beta-lactam resistance factor (fibA)	46/46	0/218	34, 251
gbs0579	dipeptidase	46/46	0/218	35, 252
gbs0580	zinc ABC transporter, zinc-binding adhesion liprot	45/45	2/235	36, 253
gbs0628	cell wall surface anchor family protein - (IPxTG)	42/44	0/219	37, 254
gbs0632	cell wall surface anchor family protein, putative (FPKTG motive)	44/45	0/238	38, 255
gbs0667	regulatory protein, putative, truncation	44/44	0/229	40, 257
gbs0672	transcriptional regulator (GntR family)	43/43	0/203	41, 258
gbs0687	unknown proteins	45/45	0/149	42, 259
gbs0785	Similar to penicillin binding protein 2B	45/45	0/218	43, 260
gbs0828	unknown proteins	46/46	1/120	45, 262
gbs0851	hypothetical protein	46/46	0/140	46, 263
gbs0865	gbs0865 Unknown	44/44	0/241	47, 264
gbs0890	exonuclease RexB (rexB)	46/46	0/232	48, 265
gbs0896	similar to acetoin dehydrogenase	46/46	0/239	49, 266
gbs0898	acetoin dehydrogenase, thymine PPi dependent	45/45	0/180	50, 267
gbs0904	phosphoglucomutase/phosphomannomutase family prote	46/46	0/169	51, 268
gbs0918	weakly similar to histidine triad protein, putative lipoprotein	45/45	1/209	52, 269
gbs0931	pyruvate kinase	46/46	0/185	53, 270
gbs0947	similar to L-Lactate Dehydrogenase	46/46	0/233	54, 271
gbs0948	DNA gyrase, A subunit (gyrA)	44/44	0/172	55, 272
gbs1035	conserved hypothetical protein	46/46	0/210	69, 286
gbs1066	gbs1066 Unknown	17/46	2/92	71, 288
gbs1087	highly repetitive peptidoglycan bound protein (LPXTG motif)	42/45	n.d.	72, 289

ORF	Common name	Gene distribution (present of 50)	Amino acid substitutions (in serotype IA strain)*	Seq. ID (DNA, Prot.)
gbs1103	ABC transporter (ATP-binding protein)	46/46	1/165	73, 290
gbs1116	xanthine permease (pbuX)	45/45	1/170	74, 291
gbs1158	Similar to oxidoreductase	44/44	1/170	78, 295
gbs1165	cysteine desulphurase (iscS-1)	43/43	0/148#	79, 296
gbs1195	staphylokinase and streptokinase	45/45	60/142	80, 297
gbs1209	ATP-dependent DNA helicase PcrA	43/44	1/94#	81, 298
gbs1214	conserved hypothetical protein	43/46	0/97	82, 299
gbs1260	ABC transporter, ATP-binding protein	44/46	1/198	84, 301
gbs1306	Laminin binding protein (Spellerberg,B et al 1999)	45/46	0/215	87, 304
gbs1307	lmb laminin-binding surface protein	45/45	n.d.	88, 305
gbs1308	C5a peptidase, authentic frameshift	46/46	0/205	89, 306
gbs1309	hypothetical protein	44/46	0/214	90, 307
gbs1356	Putative peptidoglycan linked protein (LPXTG motif) - Agglutinin receptor	20/46	50/211#	94, 311
gbs1376	similar to ATP-dependent Clp proteinase (ATP-binding subunit), ClpL	45/45	0/197	95, 312
gbs1377	similar to homocysteine S-methyltransferase	45/45	0/55	96, 313
gbs1386	-hydroxy-3-methylglutaryl-coenzyme A synthase	44/44	0/219	97, 314
gbs1390	gbs1390 Unknown	43/43	0/198	98, 315
gbs1391	gbs1391 Unknown	44/44	0/214	99, 316
gbs1403	similar to 5'-nucleotidase, putative peptidoglycan bound protein (LPXTN)	45/45	3/189	100, 317
gbs1408	Similar to ABC transporter (ATP-binding protein)	45/45	0/205	101, 318
gbs1429	unknown proteins	46/46	1/193	103, 320
gbs1452	rplT 50S ribosomal protein L20	46/46	0/101	105, 322
gbs1464	ferrichrome ABC transporter (permease	44/44	2/232	106, 323
gbs1470	conserved hypothetical protein	46/46	2/164	107, 324
gbs1528	conserved hypothetical protein	45/45	0/213	108, 325
gbs1531	UvrB excinuclease ABC chain B	45/45	0/108	110, 327
gbs1533	glutamine ABC transporter, glutamine-binding prote	44/44	0/166	111, 328

ORF	Common name	Gene distribution (present of 50)	Amino acid substitutions (in serotype IA strain)*	Seq. ID (DNA, Prot.)
gbs1542	oxidoreductase, aldo/keto reductase family	45/45	1/219	113, 330
gbs1565	hypothetical protein	43/43	1/218	115, 332
gbs1586	peptidyl-prolyl cis-trans isomerase, cyclophilin-type	45/45	1/227	116, 333
gbs1591	5-methylthioadenosine nucleosidase/S-adenosylhomoc	45/45	0/203	117, 334
gbs1632	similar to branched-chain amino acid ABC transporter, amino acid-binding protein	45/45	0/223	118, 335
gbs1638	amino acid permease	45/45	0/100	119, 336
gbs1662	conserved hypothetical protein	45/45	0/213	120, 337
gbs1666	SWI/SNF family helicase	45/45	0/200	121, 338
gbs1673	conserved hypothetical protein	45/45	0/147	122, 339
gbs1695	dihydroxyacetone kinase family protein	43/43	1/165	123, 340
gbs1754	excinuclease ABC, A subunit (uvrA)	43/43	0/224	124, 341
gbs1760	Similar to A/G-specific adenine glycosylase	46/46	0/181	125, 342
gbs1777	glycerol uptake facilitator protein, putative	43/43	0/199	126, 343
gbs1783	polyprenyl synthetase family protein	45/45	0/217	127, 344
gbs1784	ABC transporter, ATP-binding protein CydC	45/45	1/220	128, 345
gbs1790	unknown proteins	41/43	3/75#	129, 346
gbs1805	Similar to secreted unknown proteins	45/45	0/66	130, 347
gbs1816	HD domain protein	43/43	1/176	131, 348
gbs1821	Similar to 23S ribosomal RNA methyltransferase	43/43	2/155#	132, 349
gbs1834	two-component sensor histidine kinase	44/44	0/213	134, 351
gbs1842	transcriptional antiterminator, BglG family	43/43	0/208	135, 352
gbs1850	hypothetical transaldolase	44/44	0/194	136, 353
gbs1875	alkyl hydroperoxide reductase (large subunit) and NADH dehydrogenase	46/46	0/192	138, 355
gbs1879	endopeptidase O (pepO)	43/43	0/135	139, 356
gbs1893	2-keto-3-deoxygluconate kinase	36/46	0/228	140, 357
gbs1899	N-acetylmuramoyl-L-alanine amidase, family 4 prote	43/43	0/149	141, 358
gbs1907	citrate carrier protein, CCS family	43/43	0/219	142, 359
gbs1925	similar to pneumococcal histidine triad protein B precursor (N-terminal part)	43/43	0/103	144, 361
gbs1962	conserved hypothetical protein	28/46	0/136	145, 362
gbs2008	similar to C5A peptidase, putative	43/43	n.d.	146, 363

ORF	Common name	Gene distribution (present of 50)	Amino acid substitutions (in serotype IA strain)*	Seq. ID (DNA, Prot.)
	peptidoglycan linked protein (LPXTG motif)			
gbs2018	putative peptidoglycan linked protein (LPXTG motif)	43/45	0/104	147, 364
gbs2029	unknown proteins	44/44	1/238	148, 365
gbs2054	DNA mismatch repair protein HexA	46/46	0/206	150, 367
gbs2060	aspartyl-tRNA synthetase (aspS)	46/46	2/211	152, 369
gbs2106	protein of unknown function/lipoprotein, putative	44/44	0/160	154, 371
gbs2118	similar to inosine monophosphate dehydrogenase	43/43	0/113	155, 372
gbs2131	ABC transporter, permease protein, putative	45/45	0/237	156, 373

Table 4. Immunogenicity of epitopes in mice

ORF	aa from	aa to	IB	Peptide ELISA	Seq ID
gbs0016	110	129	+		219
	168	184	+		
gbs0986	877	901	+		277
	333	354	+		
	326	344	+		
	801	809	+		
gbs1805	1	54		+++++	347
gbs2018	544	563	++	+++++	364
	31	51	+	+++	
	107	119	+		
	399	417	++	++++	
	503	519	+	++++	
gbs0012	120	198	++	+	218
gbs0016	20	35	+	++	219
gbs0031	118	201		++++	221
gbs0428	48	132	+	+++++	242
gbs0538	118	136	+	++++	249
gbs0580	162	178	+	+	253
gbs0628	347	364	+	+++++	254
gbs0632	699	715	+	+++++	255
gbs0672	50	76		+	258
gbs0918	785	819	+	+++++	269
	44	128	++		
gbs0971	90	128		+++++	274
gbs1087	314	384	+		289
gbs1143	327	349		+++	293
gbs1306	242	314	++	++++	304
	405	478	++		
	23	100	+		
gbs1307	129	210	++		305
gbs1309	162	188		++	307
gbs1352	750	772	++	+++++	310
gbs1632	1	56		++	335
gbs1662	322	337	+	+++++	337
gbs1673	72	90	+	+++++	339
gbs1784	374	395		+	345
gbs1816	136	159	+	++++	348
gbs1899	141	164	+		358
gbs1925	96	157	++	+	361
gbs2008	1	82		+	363
gbs2018	489	556	+	+++++	364
gbs2106	159	183		++	371
	49	133	+	+++++	

Table 5: Immunogenic proteins identified by bacterial surface display.

S. agalactiae antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immunogenic region (aa)	Seq. ID (DNA, Prot.)
gbs0233	similar to glycine betaine/carnitine/choline ABC transporter (osmoprotectant-binding protein)	4-26, 35-41, 53-61, 73-84, 103-108, 114-120, 140-146, 156-162, 192-208, 214-219, 227-233, 239-252, 260-268, 284-297	A:5, B:9, H:3	1-48, 113-133	463, 475
gbs0419	protein of unknown function/lipoprotein	4-27, 38-44, 50-56, 59-64, 72-79, 83-89, 92-97, 108-116, 123-148, 152-167, 183-196, 200-220, 232-244, 255-261, 265-274, 282-302, 309-317	C:3, D:4, J:2, K:4	1-79, 231-302	464, 476
gbs0456	cell wall surface anchor family protein	6-28, 66-72, 85-105, 115-121, 144-151, 160-170, 176-185, 223-230, 252-288, 296-310, 319-333, 367-374, 458-464, 471-480, 483-488, 520-528, 530-549, 559-564, 593-601, 606-616, 636-643, 655-662, 676-682, 684-699, 719-726, 735-750, 757-764, 777-785, 799-810, 812-843, 846-853, 868-873, 880-889, 891-899, 909-929, 934-940, 963-969, 998-1004, 1007-1014, 1016-1022, 1030-1046	A:9, B:125, F:9	1-80, 808-821	465, 477
gbs0942	protein of unknown function/lipoprotein	7-24, 35-41, 75-81, 91-114, 122-132, 137-144, 148-156, 183-192, 194-200, 212-228, 233-238, 251-258, 275-295, 326-332, 337-346	A:3, B:9, C:2, J:3	1-79, 305-321	466, 478
gbs0973	gid protein (gid)	31-38, 42-52, 66-72, 86-92, 98-104, 115-122, 127-146, 154-164, 169-187, 198-212, 225-237, 255-269	D:2, G:3, H:8, I:2, K:2	13-92, 135-142	467, 479
gbs0975	Unknown protein	4-36, 39-49, 63-69, 71-77, 81-88, 123-131, 133-139, 160-169, 174-180, 188-194, 210-217, 273-278, 289-300, 317-334, 336-341, 383-401, 425-438	A:3, B:5, C:5, D:2, J:2	1-68, 212-270, 402-446	468, 480
gbs1038	permease, putative	21-29, 31-42, 49-63, 72-79, 81-93, 112-132, 159-165, 188-195, 197-232, 262-267, 279-286, 294-301, 318-326, 348-366, 381-405, 409-426, 436-465, 471-480, 484-492, 497-505, 521-544, 554-561, 567-577, 581-589, 601-609, 611-622, 636-651, 653-667, 669-685, 700-708, 716-722, 729-744, 749-766, 780-786, 789-811, 814-864	B:2, C:3, E:3, J:2	1-57, 84-106	469, 481
gbs1144	Unknown protein	6-24, 35-48, 57-63, 72-78, 87-92, 113-119, 123-137, 147-153, 173-181, 212-233	C:30	1-124	470, 482
gbs1279	conserved domain protein	13-34, 62-69, 78-83, 86-91, 98-104, 107-115, 146-159, 179-188, 195-205, 209-221, 226-233, 239-253, 276-282, 284-294, 297-308, 331-354, 375-382, 388-399, 421-433,	A:44, B:2, C:4	1-84	471, 483

S. agalactiae antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immunogenic region (aa)	Seq. ID (DNA, Prot.)
		449-458, 464-469, 472-491, 508-513, 525-531, 534-550, 575-593, 601-618, 629-635, 654-661, 666-680, 706-721, 723-740, 771-805, 810-830, 845-851			
gbs1441	conserved hypothetical protein	4-32, 45-64, 73-83, 86-92, 100-111, 125-147, 157-163, 170-175, 177-188, 226-232, 245-252, 258-274, 320-335, 348-359	A:2, B:8, C:2	1-71	472, 484
gbs1677	sodium transport family protein	13-40, 43-71, 76-83, 87-101, 109-119, 125-156, 162-175, 182-219, 226-232, 240-262, 270-287, 306-318, 326-342, 344-408, 414-444, 449-456	B:3, C:2, K:5	1-51	473, 485
gbs2093	Membrane protein, putative	4-16, 18-34, 45-54, 99-108, 134-140, 203-212, 241-257, 266-274, 279-291, 308-315, 330-336, 355-370, 374-382, 402-410, 428-455, 466-472, 474-480, 531-554, 560-566, 572-580, 597-618, 632-660, 664-674, 676-685, 691-705, 708-735, 750-768	B :5, C :8, D :2	1-87, 342-480	474, 486

Table 6. Immune reactivity of epitopes within identified antigens with human sera

Peptides	Positivity	aa from	aa to	Seq ID
gbs0233.1	+	115	132	475
gbs0233.2	+	1	26	475
gbs0419.1	++	33	55	476
gbs0942.2	+++	1	25	478
gbs0973.1	+	37	61	479
gbs0975.1	+	1	24	480
gbs1038.1	+	1	23	481
gbs1144.3	++	46	60	482
gbs1279.1	+++	1	28	483
gbs1279.2	+	23	50	483
gbs1279.3	++	45	71	483
gbs1441.1	+	1	22	484
gbs1441.2	++	17	38	484
gbs1677.1	+	1	22	485
gbs1677.2	+	17	38	485
gbs2093.1	+	1	27	486
gbs2093.2	++	22	47	486
gbs2093.4	+	422	447	486

Table 7. Immunogenicity of epitopes in mice

ORF	aa from	aa to	Peptide ELISA	Seq ID
gbs0233	115	132	++++	475
	1	47	++	
gbs0419	1	55	++++	476
gbs0456	22	85	++	477
gbs0942	307	320	+	478
	1	44	++	
gbs0973	15	76	++	479
	40	92	+++	
gbs0975	1	59	++	480
	213	269	+	
	403	445	+	
gbs1038	1	56	++++	481
	85	105	+	
gbs1144	37	121	+++++	482
gbs1279	1	71	+++++	483
gbs1441	1	38	+++	484
gbs1677	1	38	+++	485
gbs2093	1	47	+++	486

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Claims:

1. An isolated nucleic acid molecule encoding a hyperimmune serum reactive antigen or a fragment thereof comprising a nucleic acid sequence, which is selected from the group consisting of:
 a) a nucleic acid molecule having at least 70% sequence identity to a nucleic acid molecule selected from **Seq ID No** 14, 90, 157-216,
 b) a nucleic acid molecule which is complementary to the nucleic acid molecule of a),
 c) a nucleic acid molecule comprising at least 15 sequential bases of the nucleic acid molecule of a) or b)
 d) a nucleic acid molecule which anneals under stringent hybridisation conditions to the nucleic acid molecule of a), b), or c)
 e) a nucleic acid molecule which, but for the degeneracy of the genetic code, would hybridise to the nucleic acid molecule defined in a), b), c) or d).

2. The isolated nucleic acid molecule according to claim 1, wherein the sequence identity is at least 80%, preferably at least 95%, especially 100%.

3. An isolated nucleic acid molecule encoding a hyperimmune serum reactive antigen or a fragment thereof comprising a nucleic acid sequence selected from the group consisting of
 a) a nucleic acid molecule having 96 % or more than 96 %, preferably at least 98 %, especially 100 % sequence identity to a nucleic acid molecule selected from **Seq ID No** 1, 3, 5-13, 15, 18-25, 27-31, 33-36, 39-68, 70-85, 92-100, 103-126, 128-145, 147, 149-156, 217, 435-448 and 463-474.
 b) a nucleic acid molecule which is complementary to the nucleic acid molecule of a),
 c) a nucleic acid molecule comprising at least 15 sequential bases of the nucleic acid molecule of a) or b)
 d) a nucleic acid molecule which anneals under stringent hybridisation conditions to the nucleic acid molecule of a), b) or c),
 e) a nucleic acid molecule which, but for the degeneracy of the genetic code, would hybridise to the nucleic acid defined in a), b), c) or d).

4. An isolated nucleic acid molecule encoding a hyperimmune serum reactive antigen or a fragment thereof comprising a nucleic acid sequence selected from the group consisting of
 a) a nucleic acid molecule having 98 % or more than 98%, escpecially 100 % sequence identity to a nucleic acid molecule selected from **Seq ID No** 32, 86, 91, 101, 127,
 b) a nucleic acid molecule which is complementary to the nucleic acid of a),
 c) a nucleic acid molecule which, but for the degeneracy of the genetic code, would hybridise to the nucleic acid defined in a), b), c) or d).

5. The nucleic acid molecule according to any one of the claims 1, 2, 3 or 4, wherein the nucleic acid is DNA.

6. The nucleic acid molecule according to any one of the claims 1,2, 3, 4, or 5 wherein the nucleic acid is RNA.

7. An isolated nucleic acid molecule according to any one of claims 1 to 5, wherein the nucleic acid molecule is isolated from a genomic DNA, especially from a *S. agalactiae* genomic DNA.

8. A vector comprising a nucleic acid molecule according to any one of claims 1 to 7.

9. A vector according to claim 8, wherein the vector is adapted for recombinant expression of the hyperimmune serum reactive antigens or fragment thereof encoded by the nucleic acid molecule according to any one of claims 1 to 7.

10. A host cell comprising the vector according to claim 8 or 9.

11. A hyperimmune serum-reactive antigen comprising an amino acid sequence being encoded by a nucleic acid molecule according to any one of the claims 1, 2, 5, 6 or 7 and fragments thereof, preferably the amino acid sequence is selected from the group consisting of **Seq ID No** 231, 307, 374-433.

12. A hyperimmune serum-reactive antigen comprising an amino acid sequence being encoded by a nucleic acid molecule according to any one of the claims 3, 5, 6, or 7 and fragments thereof, preferably the amino acid sueqnece is selected from the group consisting of **Seq ID No** 218, 220, 222-230, 232, 235-242, 244-248, 250-253, 256-285, 287-302, 309-317, 320-343, 345-362, 364, 366-373, 434, 449-462 and 475-486.

13. A hyperimmune serum-reactive antigen comprising an amino acid sequence being encoded by a nucleic acid molecule according to any one of the claims 4, 5, 6, or 7 and fragments thereof, preferably the amino acid sequence is selected from the group consisting of **Seq ID No** 249, 303, 308, 318, 344.

14. Fragments of hyperimmune serum-reactive antigens selected from the group consisting of peptides comprising amino acid sequences of column "predicted immunogenic aa" and "location of identified immunogenic region" of Table 1**A, especially peptides comprising amino acid** 4-20, 35-44, 65-70, 73-87, 92-98, 112-137, 152-161, 177-186, 193-200, 206-213, 229-255, 282-294, 308-313, 320-326, 349-355, 373-384, 388-406, 420-425 and 115-199 of **Seq ID No** 218; 5-24, 35-41, 44-70, 73-89, 103-109, 127-143, 155-161, 185-190, 192-207, 212-219, 246-262, 304-336, 372-382, 384-393, 398-407, 412-418, 438-444, 1-75, 76-161 and 164-239 of **Seq ID No** 219; 4-10, 16-58, 60-71, 77-92, 100-126, 132-146, 149-164, 166-172, 190-209, 214-220, 223-229, 241-256, 297-312, 314-319, 337-343, 351-359, 378-387, 398-418, 421-428, 430-437, 440-448, 462-471, 510-519, 525-536, 552-559, 561-568, 573-582, 596-602, 608-630, 637-649, 651-665, 681-702, 714-732, 739-745, 757-778, 790-805, 807-815, 821-829, 836-842, 846-873, 880-903, 908-914, 916-923, 931-940, 943-948, 956-970, 975-986, 996-1015, 1031-1040, 1051-1069, 1072-1095, 1114-1119, 1130-1148, 1150-1157, 1169-1176, 1229-1238 and 802-812 of **Seq ID No** 220; 5-12, 14-26, 35-47, 52-67, 72-78, 83-98, 121-141, 152-159, 163-183, 186-207, 209-257, 264-277, 282-299, 301-309, 312-318, 324-339, 358-368, 372-378, 387-397, 425-431 and 46-291 of **Seq ID No** 221; 29-38, 44-64, 70-76, 78-87, 94-100, 102-112, 119-134, 140-149, 163-173, 178-186, 188-194, 207-234, 247-262, 269-290 and 73-92 of **Seq ID No** 222; 10-28, 36-63, 77-87, 103-119, 127-136, 141-169, 171-183, 195-200, 207-232, 236-246, 251-265, 268-283, 287-297, 314-322, 335-343, 354-363, 384-390, 405-411, 419-436, 443-455, 467-473, 480-513, 518-529, 550-557, 565-585, 602-608, 616-625, 632-660, 665-677, 685-701, 726-736, 738-747, 752-761, 785-796, 801-813, 838-853, 866-871 and 757-774 of **Seq ID No** 223; 31-38, 61-66, 74-81, 90-115, 123-145, 154-167, 169-179, 182-193, 200-206, 238-244, 267-272 and 235-251 of **Seq ID No** 224; 19-25, 38-54, 56-64, 66-72, 74-92, 94-100, 116-129, 143-149, 156-183, 204-232, 253-266, 269-275, 294-307 and 241-313 of **Seq ID No** 225; 5-34, 50-56, 60-65, 74-85, 89-97, 108-119, 159-165, 181-199, 209-225, 230-240, 245-251, 257-262, 274-282, 300-305 and 64-75 of **Seq ID No** 226; 5-13, 16-21, 27-42, 45-52, 58-66, 74-87, 108-114, 119-131 and 39-51 of **Seq ID No** 227; 6-23, 46-54, 59-65, 78-84, 100-120, 128-133, 140-146, 159-165, 171-183, 190-204, 224-232, 240-248, 250-259, 274-280, 288-296, 306-315 and 267-274 of **Seq ID No** 228; 5-12, 15-24, 26-36, 42-65, 68-80, 82-104, 111-116, 125-144, 159-167, 184-189, 209-218, 235-243, 254-265, 269-283, 287-300, 306-316, 318-336, 338-352, 374-392 and 162-174 of **Seq ID No** 229; 30-42, 45-54 and 25-37 of **Seq ID No** 230; 10-30, 53-59, 86-95, 116-130, 132-147, 169-189, 195-201, 212-221, 247-256, 258-265, 278-283, 291-298, 310-316, 329-339, 341-352, 360-367, 388-396, 398-411, 416-432, 443-452, 460-466, 506-512, 515-521, 542-548 and 419-431 of **Seq ID No** 231; 4-27, 30-53, 60-67, 70-90, 92-151, 159-185, 189-195, 198-210, 215-239 and 173-189 of **Seq ID No** 232; 4-26, 41-54, 71-78, 116-127, 140-149, 151-158, 161-175, 190-196, 201-208, 220-226, 240-252, 266-281, 298-305,

308-318, 321-329, 344-353, 372-378, 384-405, 418-426, 429-442, 457-463, 494-505, 514-522 and 174-188 of **Seq ID No** 233; 17-25, 27-39, 61-67, 81-89, 99-110, 120-131, 133-139, 147-161, 167-172, 179-185, 192-198, 203-213, 226-238, 243-258, 261-267, 284-290, 296-307, 311-328, 340-352, 356-371 and 239-256 of **Seq ID No** 234; 8-30, 40-49, 67-80, 114-123, 126-142, 152-162, 188-194 and 57-70 of **Seq ID No** 235; 4-23, 28-34, 36-47, 50-61, 76-81, 89-94, 96-104, 112-119, 126-146, 155-181, 195-200, 208-214, 220-229, 244-260, 263-276, 282-288, 292-300, 317-323, 336-351, 353-359, 363-375, 382-399, 415-432, 444-455, 458-471, 476-481, 484-492, 499-517, 522-529, 535-541, 543-568, 572-584, 586-600, 607-617, 626-637, 656-675 and 282-297 of **Seq ID No** 236; 6-24, 30-35, 38-45, 63-91, 134-140, 146-160, 167-188, 214-220, 226-234, 244-250, 260-270, 286-301, 316-329, 340-371, 429-446, 448-459, 474-481, 485-491, 512-526, 537-544, 550-565, 573-583, 596-613, 621-630, 652-658 and 87-97 of **Seq ID No** 237; 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4-15 and 5-23 of **Seq ID No** 401; 10-21 of **Seq ID No** 402; 6-16 of **Seq ID No** 403; 4-29, 31-38 and 2-14 of **Seq ID No** 404; 4-35 and 33-42 of **Seq ID No** 405; 2-17 of **Seq ID No** 406; 9-18, 30-35 and 15-33 of **Seq ID No** 407; 4-9 and 6-12 of **Seq ID No** 408; 3-17 of **Seq ID No** 409; 12-21, 37-44, 52-61, 72-80 and 38-48 of **Seq ID No** 410; 4-10, 29-44, 54-61, 69-78 and 13-27 of **Seq ID No** 411; 13-23, 36-53 and 2-15 of **Seq ID No** 412; 4-25, 28-46, 56-72, 81-99, 120-132, 134-142, 154-160 and 129-141 of **Seq ID No** 413; 4-15, 24-33, 35-41, 64-86 and 21-33 of **Seq ID No** 414; 9-15 and 4-13 of **Seq ID No** 415; 4-11, 13-19, 34-48 and 15-32 of **Seq ID No** 416; 4-21 and 11-31 of **Seq ID No** 417; 23-57 and 38-

50 of **Seq ID No** 418; 4-32 and 3-13 of **Seq ID No** 419; 4-10, 13-25, 32-42, 56-68, 72-84 and 26-38 of **Seq ID No** 420; 4-20, 31-48, 52-58, 65-71, 80-93, 99-108, 114-123 and 37-49 of **Seq ID No** 421; 6-12, 14-20 and 3-25 of **Seq ID No** 422; 14-25, 27-38 and 5-14 of **Seq ID No** 423; 4-41, 57-105, 109-118, 123-136, 144-152 and 86-99 of **Seq ID No** 424; 6-19 of **Seq ID No** 425; 2-19 of **Seq ID No** 426; 14-47 and 1-14 of **Seq ID No** 427; 4-21, 29-44 and 2-18 of **Seq ID No** 428; 23-29 and 10-28 of **Seq ID No** 429; 6-16, 22-36 and 11-22 of **Seq ID No** 430; 4-19, 30-44 and 18-27 of **Seq ID No** 431; 5-15, 37-45, 58-65 and 38-47 of **Seq ID No** 432; 4-15, 23-34 and 4-15 of **Seq ID No** 433; 30-36, 44-54, 79-85, 101-114, 138-152, 154-164, 170-175, 179-200, 213-220, 223-240, 243-255, 258-264, 268-284 and 10-28 of **Seq ID No** 434; **the peptides comprising amino acid sequences of column "Identical region" of the Table 1B, especially peptides comprising amino acid** 210-226 and 738-753 of **Seq ID No** 449; 326-344, 326-348, 338-354, 371-392, 801-809 and 877-901 of **Seq ID No** 450; 893-906 of **Seq ID No** 451; 51-69 of **Seq ID No** 452; 110-125 of **Seq ID No** 453; 291-305 of **Seq ID No** 454; 210-226 and 738-753 of **Seq ID No** 455; 326-344, 326-348, 338-354, 371-392, 801-809 and 877-901 of **Seq ID No** 456; 893-906 of **Seq ID No** 457; 51-69 of **Seq ID No** 458; 110-125 of **Seq ID No** 459; 291-305 of **Seq ID No** 460; 32-44 of **Seq ID No** 461; 399-410 of **Seq ID No** 462; the serum reactive epitopes **as specified in the column of "aa from" to "aa to"** of Table **2, especially peptides comprising amino acid** 120-143, 138-161 and 156-179 of **Seq ID No** 218; 110-129 and 168-184 of **Seq ID No** 219; 74-90 of **Seq ID No** 222; 759-773 of **Seq ID No** 223; 237-260 of **Seq ID No** 224; 265-284 of **Seq ID No** 225; 65-74 of **Seq ID No** 226; 41-50 of **Seq ID No** 227; 163-174 of **Seq ID No** 229; 26-37 of **Seq ID No** 230; 174-189 of **Seq ID No** 232; 240-256 of **Seq ID No** 234; 285-297 of **Seq ID No** 236; 238-247 of **Seq ID No** 238; 491-519 of **Seq ID No** 239; 114-140 of **Seq ID No** 243; 267-284 of **Seq ID No** 250; 439-453 of **Seq ID No** 252; 162-178 of **Seq ID No** 253; 347-364 of **Seq ID No** 254; 699-715 of **Seq ID No** 255; 60-71 of **Seq ID No** 256; 244-257 of **Seq ID No** 257; 44-63 and 57-76 of **Seq ID No** 258; 185-196 of **Seq ID No** 260; 119-129 of **Seq ID No** 263; 182-195 of **Seq ID No** 266; 32-44 and 424-442 of **Seq ID No** 267; 247-256 of **Seq ID No** 268; 678-694, 785-805, 55-77 and 72-94 of **Seq ID No** 269; 210-226 of **Seq ID No** 281; 37-59 of **Seq ID No** 289; 13-29 of **Seq ID No** 296; 136-159 of **Seq ID No** 348; 205-222 of **Seq ID No** 349; 99-110 of **Seq ID No** 350; 160-176 of **Seq ID No** 351; 457-470 of **Seq ID No** 355; 221-237 of **Seq ID No** 356; 167-190 of **Seq ID No** 357; 96-120 of **Seq ID No** 361; 399-417, 503-519 and 544-563 of **Seq ID No** 364; 46-68, 159-183 and 184-198 of **Seq ID No** 371; 463-481 of **Seq ID No** 372; **the immunogenic epitopes as specified in the column of "aa from" to "aa to" of Table 4;** especially peptides comprising amino acid 110-129 and 168-184 of **Seq ID No** 219; 877-901, 333-354, 326-344 and 801-809 of **Seq ID No** 277; 1-54 of **Seq ID No** 347; 544-563, 31-51, 107-119, 399-417 and 503-519 of **Seq ID No** 364; 120-198 of **Seq ID No** 218; 20-35 of **Seq ID No** 219; 118-201 of **Seq ID No** 221; 48-132 of **Seq ID No** 242; 118-136 of **Seq ID No** 249; 162-178 of **Seq ID No** 253; 347-364 of **Seq ID No** 254; 699-715 of **Seq ID No** 255; 50-76 of **Seq ID No** 258; 785-819 and 44-128 of **Seq ID No** 269; 90-128 of **Seq ID No** 274; 314-384 of **Seq ID No** 289; 327-349 of **Seq ID No** 293; 242-314, 405-478 and 23-100 of **Seq ID No** 304; 129-210 of **Seq ID No** 305; 162-188 of **Seq ID No** 307; 750-772 of **Seq ID No** 310; 1-56 of **Seq ID No** 335; 322-337 of **Seq ID No** 337; 72-90 of **Seq ID No** 339; 374-395 of **Seq ID No** 345; 136-159 of **Seq ID No** 348; 141-164 of **Seq ID No** 358; 96-157 of **Seq ID No** 361; 1-82 of **Seq ID No** 363; 489-556 of **Seq ID No** 364; 159-183 and 49-133 of **Seq ID No** 371; **The peptides comprising amino acid sequences of column "predicted immunogenic aa" and "location of identified immunogenic region (aa)" of Table 5, especially peptides comprising amino acid** 4-26, 35-41, 53-61, 73-84, 103-108, 114-120, 140-146, 156-162, 192-208, 214-219, 227-233, 239-252, 260-268, 284-297, 1-48 and 113-133 of **Seq ID No** 475; 4-27, 38-44, 50-56, 59-64, 72-79, 83-89, 92-97, 108-116, 123-148, 152-167, 183-196, 200-220, 232-244, 255-261, 265-274, 282-302, 309-317, 1-79 and 231-302 of **Seq ID No** 476; 6-28, 66-72, 85-105, 115-121, 144-151, 160-170, 176-185, 223-230, 252-288, 296-310, 319-333, 367-374, 458-464, 471-480, 483-488, 520-528, 530-549, 559-564, 593-601, 606-616, 636-643, 655-662, 676-682, 684-699, 719-726, 735-750, 757-764, 777-785, 799-810, 812-843, 846-853, 868-873, 880-889, 891-899, 909-929, 934-940, 963-969, 998-1004, 1007-1014, 1016-1022, 1030-1046, 1-80 and 808-821 of **Seq ID No** 477; 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of **Seq ID No** 479; 4-36, 39-49, 63-69, 71-77, 81-88, 123-131, 133-139, 160-169, 174-180, 188-194, 210-217, 273-278, 289-300, 317-334, 336-341, 383-401, 425-438, 1-68, 212-270 and 402-446 of **Seq ID No** 480; 21-29, 31-42, 49-63, 72-79, 81-93, 112-132, 159-165, 188-195, 197-232, 262-267, 279-286, 294-301, 318-326, 348-366, 381-405, 409-426, 436-465, 471-480, 484-492, 497-505, 521-544, 554-561, 567-577, 581-589, 601-609, 611-622, 636-651, 653-667, 669-685, 700-708, 716-722, 729-744, 749-766, 780-786, 789-811, 814-864, 1-57 and 84-106 of **Seq ID No** 481; 6-24, 35-48, 57-63, 72-78, 87-92, 113-119, 123-137, 147-153, 173-181, 212-233 and 1-124 of **Seq ID No** 482; 13-34, 62-69, 78-83, 86-91, 98-104, 107-115, 146-159, 179-188, 195-205, 209-221, 226-233, 239-253, 276-282, 284-294, 297-308, 331-354, 375-382, 388-399, 421-433, 449-458, 464-469, 472-491, 508-513, 525-531, 534-550, 575-593, 601-618, 629-635, 654-661, 666-680, 706-721, 723-740, 771-805, 810-830, 845-851 and 1-84 of **Seq ID No** 483; 4-32, 45-64, 73-83, 86-92, 100-111, 125-147, 157-163, 170-175, 177-188, 226-232, 245-252, 258-274, 320-335, 348-359 and 1-71 of **Seq ID No** 484; 13-40, 43-71, 76-83, 87-101, 109-119, 125-156, 162-175, 182-219, 226-232, 240-262, 270-287, 306-318, 326-342, 344-408, 414-444, 449-456 and 1-51 of **Seq ID No** 485; 4-16, 18-34, 45-54, 99-108, 134-140, 203-212, 241-257, 266-274, 279-291, 308-315, 330-336, 355-370, 374-382, 402-410, 428-455, 466-472, 474-480, 531-554, 560-566, 572-580, 597-618, 632-660, 664-674, 676-685, 691-705, 708-735, 750-768, 1-87 and 342-480 of **Seq ID No** 486; **The serum reactive epitopes as specified in the column of "aa from" to "aa to" of Table 6, especially peptides comprising amino acid** 115-132 and 1-26 of **Seq ID No** 475; 33-55 of **Seq ID No** 476; 1-25 of **Seq ID No** 478; 37-61 of **Seq ID No** 479; 1-24 of **Seq ID No** 480; 1-23 of **Seq ID No** 481; 46-60 of **Seq ID No** 482; 1-28, 23-50 and 45-71 of **Seq ID No** 483; 1-22 and 17-38 of **Seq ID No** 484; 1-22 and 17-38 of **Seq ID No** 485; 1-27, 22-47 and 422-447 of **Seq ID No** 486; **The immunogenic epitopes as specified in the column of "aa from" to "aa to" of Table 7, especially peptides comprising amino acid** 115-132 and 1-47 of **Seq ID No** 475; 1-55 of **Seq ID No** 476; 22-85 of **Seq ID No** 477; 307-320 and 1-44 of **Seq ID No** 478; 15-76 and 40-92 of **Seq ID No** 479; 1-59, 213-269 and 403-445 of **Seq ID No** 480; 1-56 and 85-105 of **Seq ID No** 481; 37-121 of **Seq ID No** 482; 1-71 of **Seq ID No** 483; 1-38 of **Seq ID No** 484; 1-38 of **Seq ID No** 485; 1-47 of **Seq ID No** 486.

15. A process for producing a *S. agalactiae* hyperimmune serum reactive antigen or a fragment thereof according to any one of the claims 11 to 14 comprising expressing the nucleic acid molecule according to any one of claims 1 to 7.

16. A process for producing a cell, which expresses a *S. agalactiae* hyperimmune serum reactive antigen or a fragment thereof according to any one of the claims 11 to 14 comprising transforming or transfecting a suitable host cell with the vector according to claim 8 or claim 9.

17. A pharmaceutical composition, especially a vaccine, comprising a hyperimmune serum-reactive antigen or a fragment thereof, as defined in any one of claims 11 to 14 or a nucleic acid molecule according to any one of claims 1 to 7.

18. A pharmaceutical composition, especially a vaccine, according to claim 17, characterized in that it further comprises an immunostimulatory substance, preferably selected from the group comprising polycationic polymers, especially polycationic peptides, immunostimulatory deoxynucleotides (ODNs), peptides containing at least two LysLeuLys motifs, neuroactive compounds, especially human growth hormone, alumn, Freund's complete or incomplete adjuvants or combinations thereof.

19. Use of a nucleic acid molecule according to any one of claims 1 to 7 or a hyperimmune serum-reactive antigen or fragment thereof according to any one of claims 11 to 14 for the manufacture of a pharmaceutical preparation, especially for the manufacture of a vaccine against *S. agalactiae* infection.

20. An antibody, or at least an effective part thereof, which binds at least to a selective part of the

hyperimmune serum-reactive antigen or a fragment thereof according to any one of claims 11 to 14.

21. An antibody according to claim 20, wherein the antibody is a monoclonal antibody.

22. An antibody according to claim 20 or 21, wherein said effective part comprises Fab fragments.

23. An antibody according to any one of claims 20 to 22, wherein the antibody is a chimeric antibody.

24. An antibody according to any one of claims 20 to 23, wherein the antibody is a humanized antibody.

25. A hybridoma cell line, which produces an antibody according to any one of claims 20 to 24.

26. A method for producing an antibody according to claim 20, characterized by the following steps:
- initiating an immune response in a non-human animal by administrating an hyperimmune serum-reactive antigen or a fragment thereof, as defined in any one of the claims 11 to 14, to said animal,
- removing an antibody containing body fluid from said animal, and
- producing the antibody by subjecting said antibody containing body fluid to further purification steps.

27. Method for producing an antibody according to claim 21, characterized by the following steps:
- initiating an immune response in a non-human animal by administrating an hyperimmune serum-reactive antigen or a fragment thereof, as defined in any one of the claims 12 to 15, to said animal,
- removing the spleen or spleen cells from said animal,
- producing hybridoma cells of said spleen or spleen cells,
- selecting and cloning hybridoma cells specific for said hyperimmune serum-reactive antigens or a fragment thereof,
- producing the antibody by cultivation of said cloned hybridoma cells and optionally further purification steps.

28. Use of the antibodies according to any one of claims 20 to 24 for the preparation of a medicament for treating or preventing *S. agalactiae* infections.

29. An antagonist, which binds to the hyperimmune serum-reactive antigen or a fragment thereof according to any one of claims 11 to 14.

30. A method for identifying an antagonist capable of binding to the hyperimmune serum-reactive antigen or fragment thereof according to any one of claims 11 to 14 comprising:
a) contacting an isolated or immobilized hyperimmune serum-reactive antigen or a fragment thereof according to any one of claims 11 to 14 with a candidate antagonist under conditions to permit binding of said candidate antagonist to said hyperimmune serum-reactive antigen or fragment, in the presence of a component capable of providing a detectable signal in response to the binding of the candidate antagonist to said hyperimmune serum reactive antigen or fragment thereof; and
b) detecting the presence or absence of a signal generated in response to the binding of the antagonist to the hyperimmune serum reactive antigen or the fragment thereof.

31. A method for identifying an antagonist capable of reducing or inhibiting the interaction activity of a hyperimmune serum-reactive antigen or a fragment thereof according to any one of claims 11 to

14 to its interaction partner comprising:

a) providing a hyperimmune serum reactive antigen or a hyperimmune fragment thereof according to any one of claims 11-14,
b) providing an interaction partner to said hyperimmune serum reactive antigen or a fragment thereof, especially an antibody according to any one of the claims 20 to 24,
c) allowing interaction of said hyperimmune serum reactive antigen or fragment thereof to said interaction partner to form a interaction complex,
d) providing a candidate antagonist,
e) allowing a competition reaction to occur between the candidate antagonist and the interaction complex ,
f) determining whether the candidate antagonist inhibits or reduces the interaction activities of the hyperimmune serum reactive antigen or the fragment thereof with the interaction partner.

32. Use of any of the hyperimmune serum reactive antigen or fragment thereof according to any one of claims 11 to 14 for the isolation and/or purification and/or identification of an interaction partner of said hyperimmune serum reactive antigen or fragment thereof.

33. A process for *in vitro* diagnosing a disease related to expression of the hyperimmune serum-reactive antigen or a fragment thereof according to any one of claims 11 to 14 comprising determining the presence of a nucleic acid sequence encoding said hyperimmune serum reactive antigen and fragment according to any one of claims 1 to 7 or the presence of the hyperimmune serum reactive antigen or fragment thereof according to any one of claims 11-14.

34. A process for *in vitro* diagnosis of a bacterial infection, especially a *S. agalactiae* infection, comprising analysing for the presence of a nucleic acid sequence encoding said hyperimmune serum reactive antigen and fragment according to any one of claims 1 to 7 or the presence of the hyperimmune serum reactive antigen or fragment thereof according to any one of claims 11 to 14.

35. Use of the hyperimmune serum reactive antigen or fragment thereof according to any one of claims 11 to 14 for the generation of a peptide binding to said hyperimmune serum reactive antigen or fragment thereof, wherein the peptide is selected from the group comprising anticalines.

36. Use of the hyperimmune serum-reactive antigen or fragment thereof according to any one of claims 11 to 14 for the manufacture of a functional nucleic acid, wherein the functional nucleic acid is selected from the group comprising aptamers and spiegelmers.

37. Use of a nucleic acid molecule according to any one of claims 11 to 14 for the manufacture of a functional ribonucleic acid, wherein the functional ribonucleic acid is selected from the group comprising ribozymes, antisense nucleic acids and siRNA.

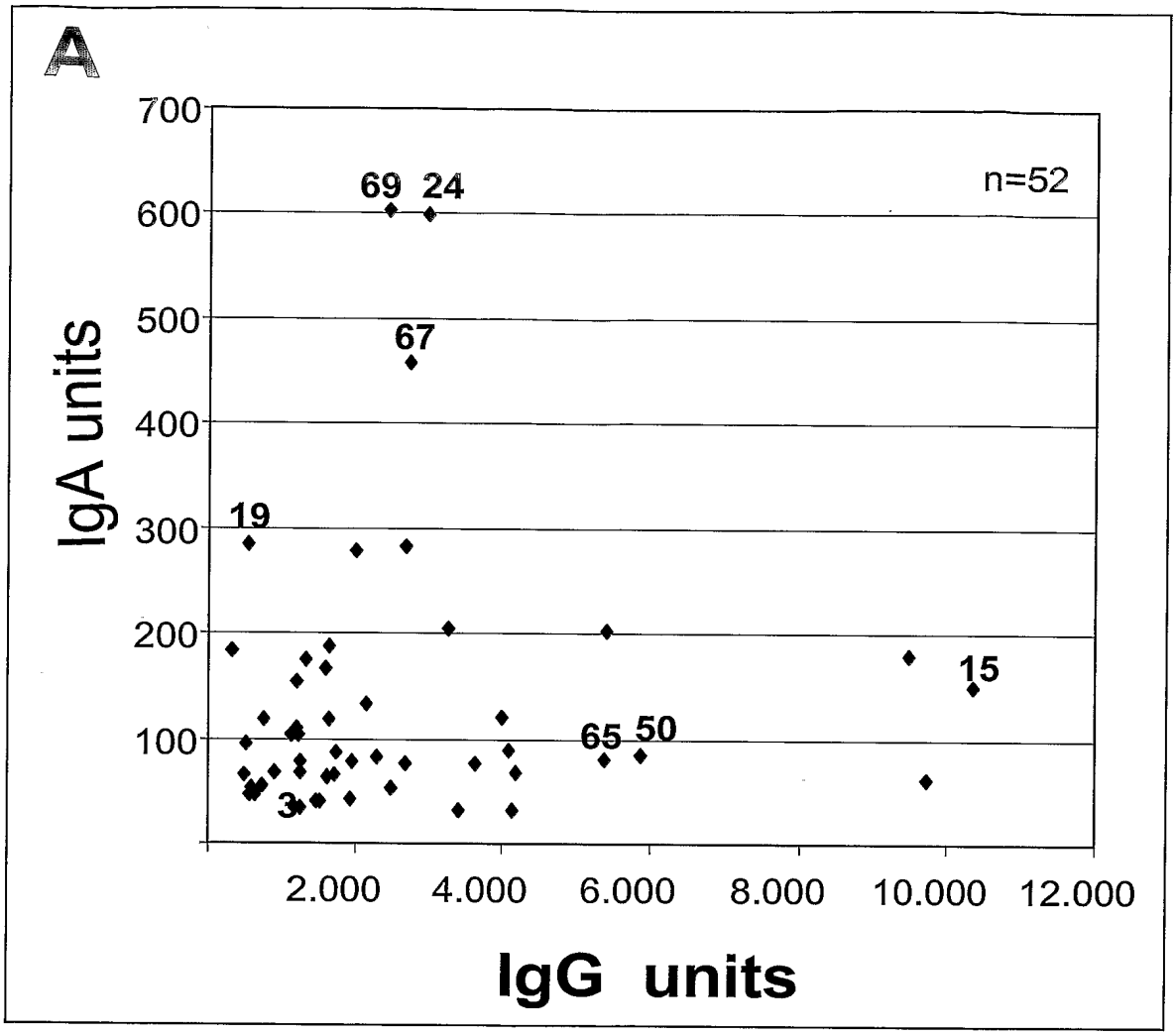
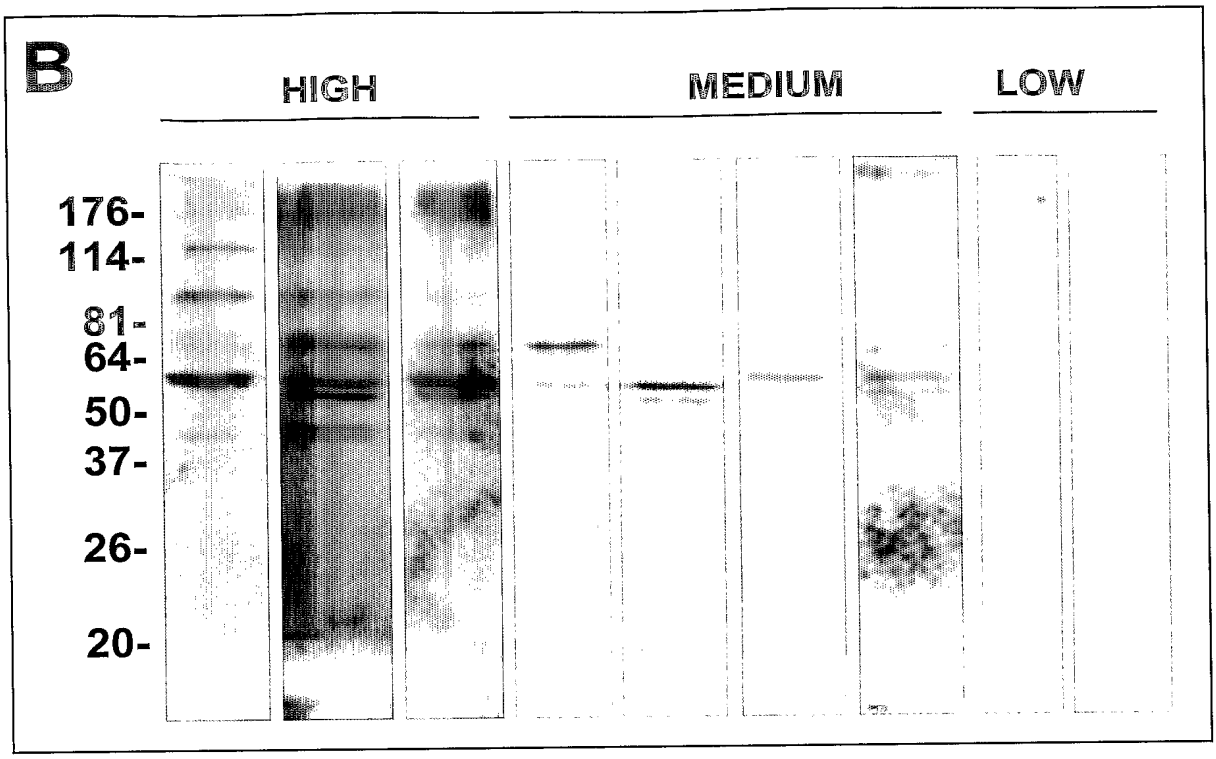

A
700
600
500
400
300
200
100
IgA units
69
24
n=52
67
19
15
65
50
3
2.000
4.000
6.000
8.000
10.000
12.000
IgG units

Fig. 1



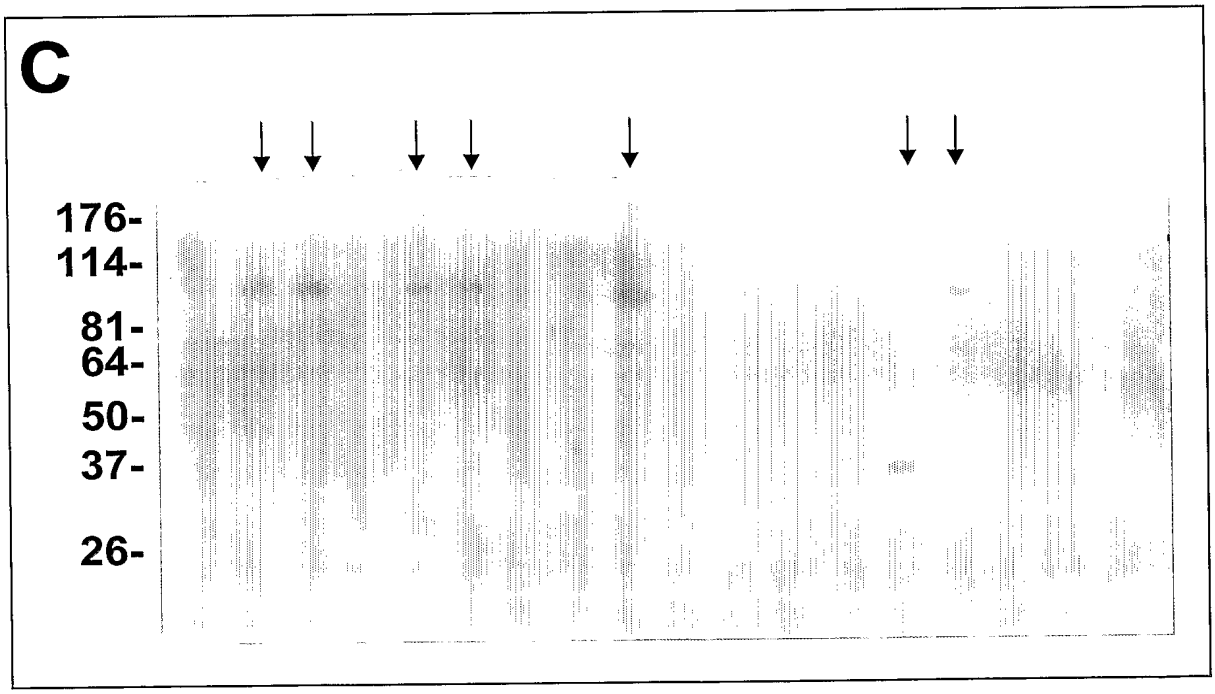
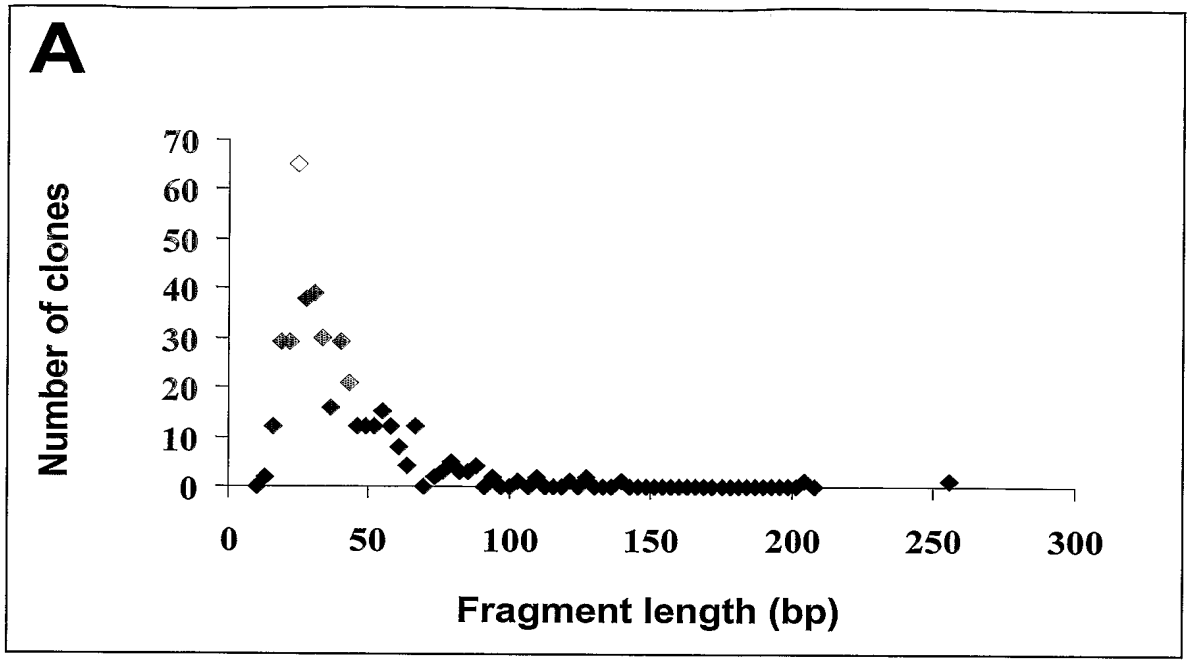
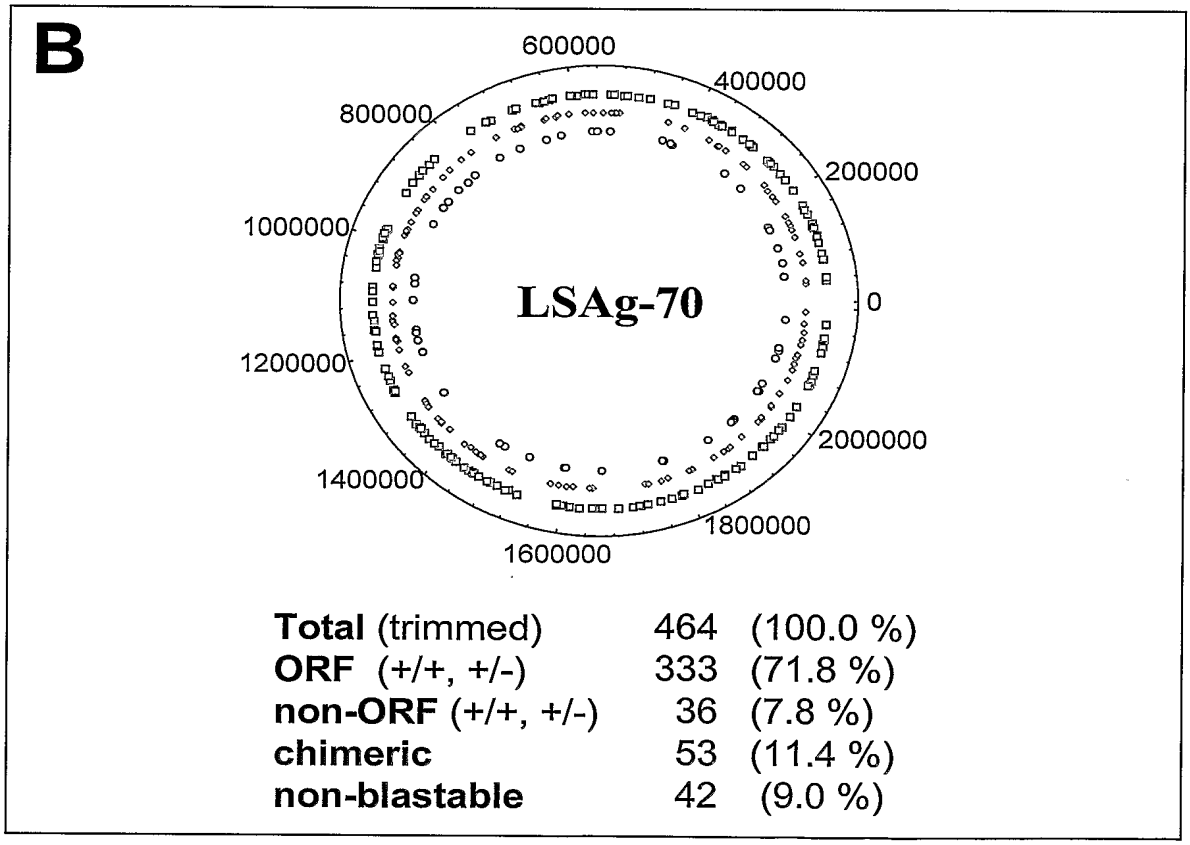


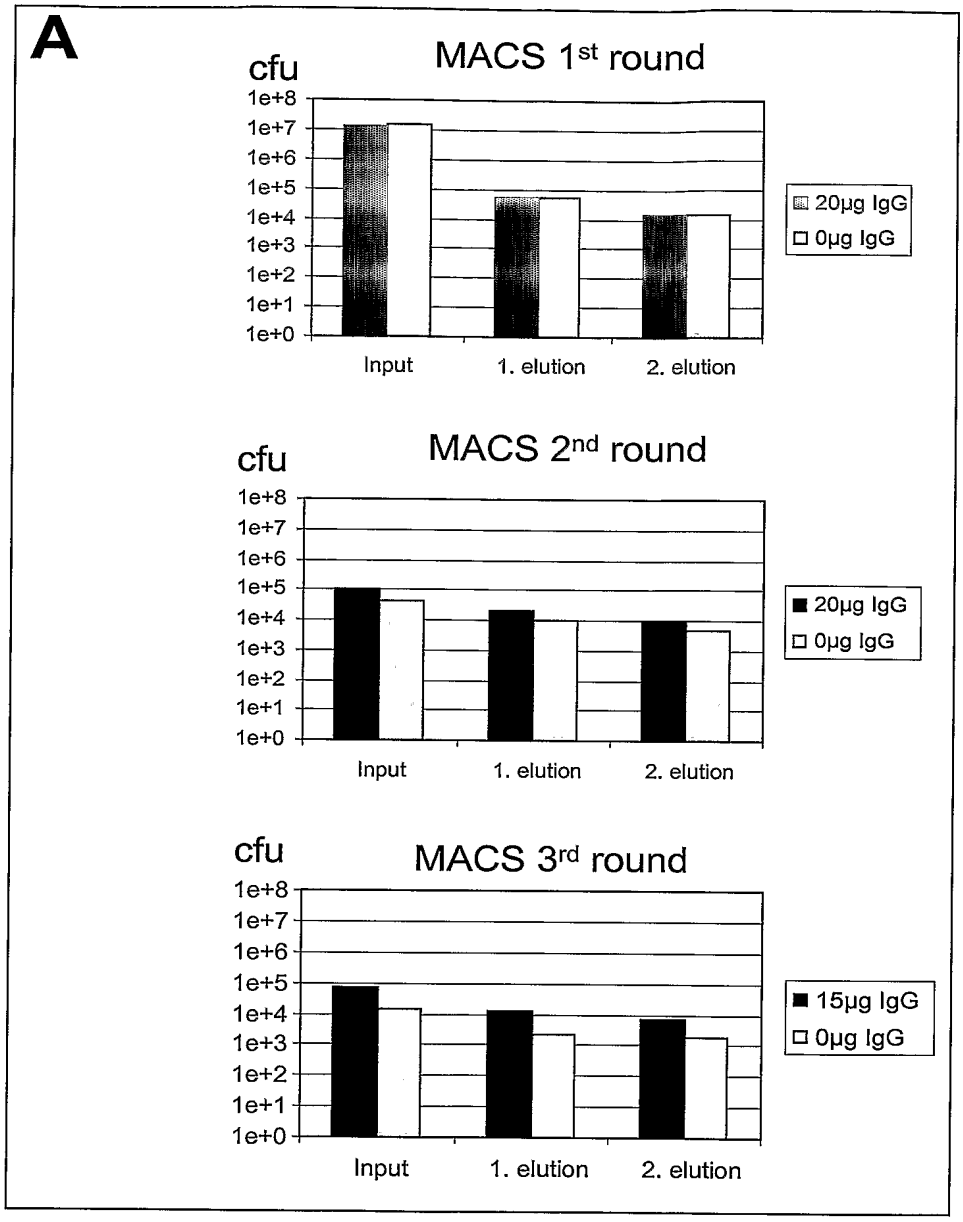
Fig. 1





Total (trimmed)	464	(100.0 %)
ORF (+/+, +/-)	333	(71.8 %)
non-ORF (+/+, +/-)	36	(7.8 %)
chimeric	53	(11.4 %)
non-blastable	42	(9.0 %)

Fig. 2


A
MACS 1st round
cfu
1e+8
1e+7
1e+6
1e+5
1e+4
1e+3
1e+2
1e+1
1e+0
Input
1. elution
2. elution
20µg IgG
0µg IgG
MACS 2nd round
cfu
20µg IgG
0µg IgG
MACS 3rd round
cfu
15µg IgG
0µg IgG

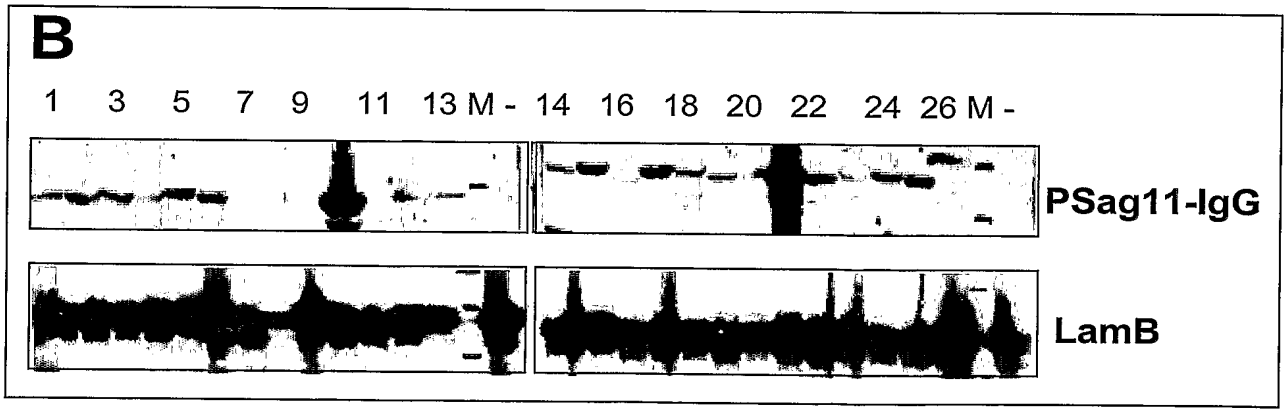
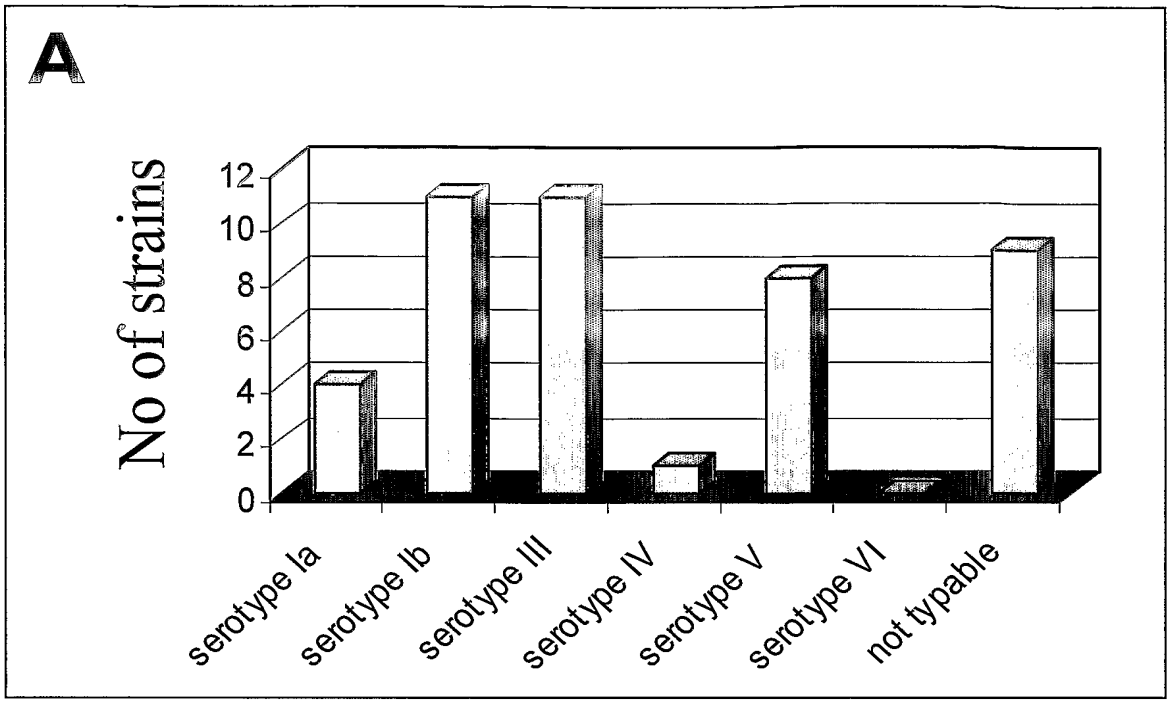

B
1 3 5 7 9 11 13 M - 14 16 18 20 22 24 26 M -
PSag11-IgG
LamB

Fig. 3


A
No of strains
12
10
8
6
4
2
0
serotype Ia
serotype Ib
serotype III
serotype IV
serotype V
serotype VI
not typable

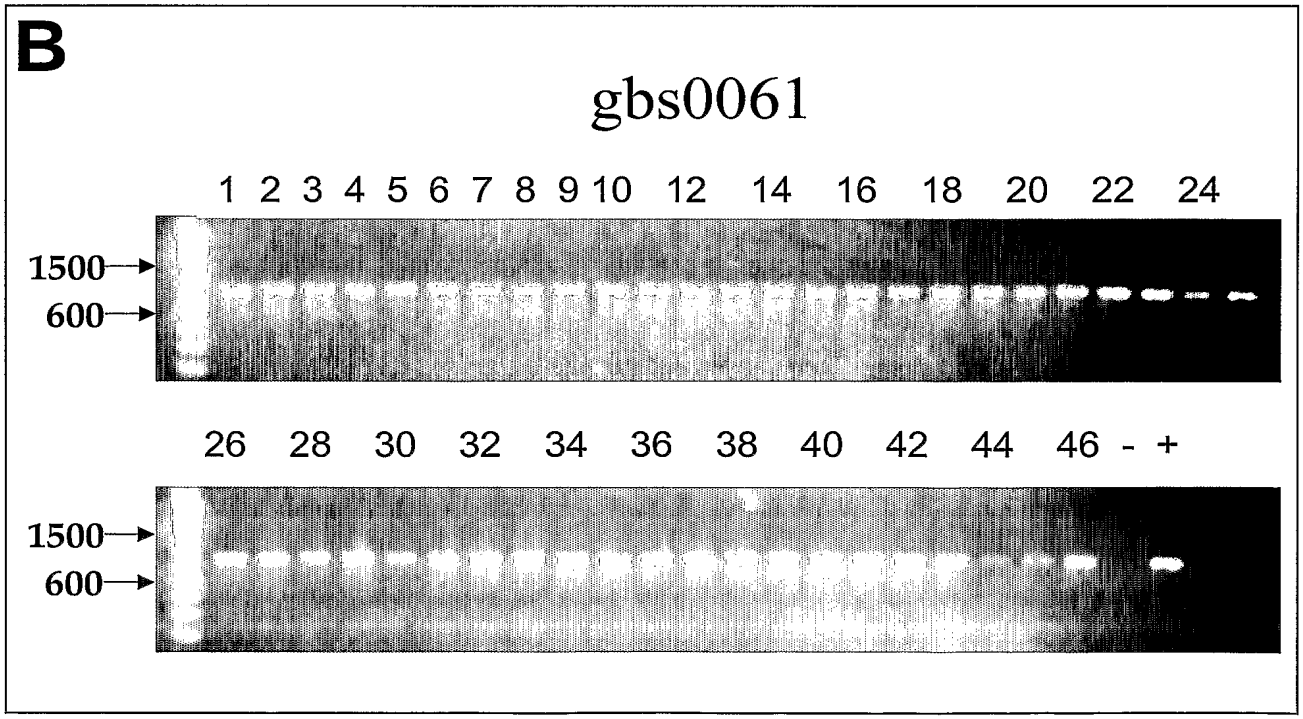
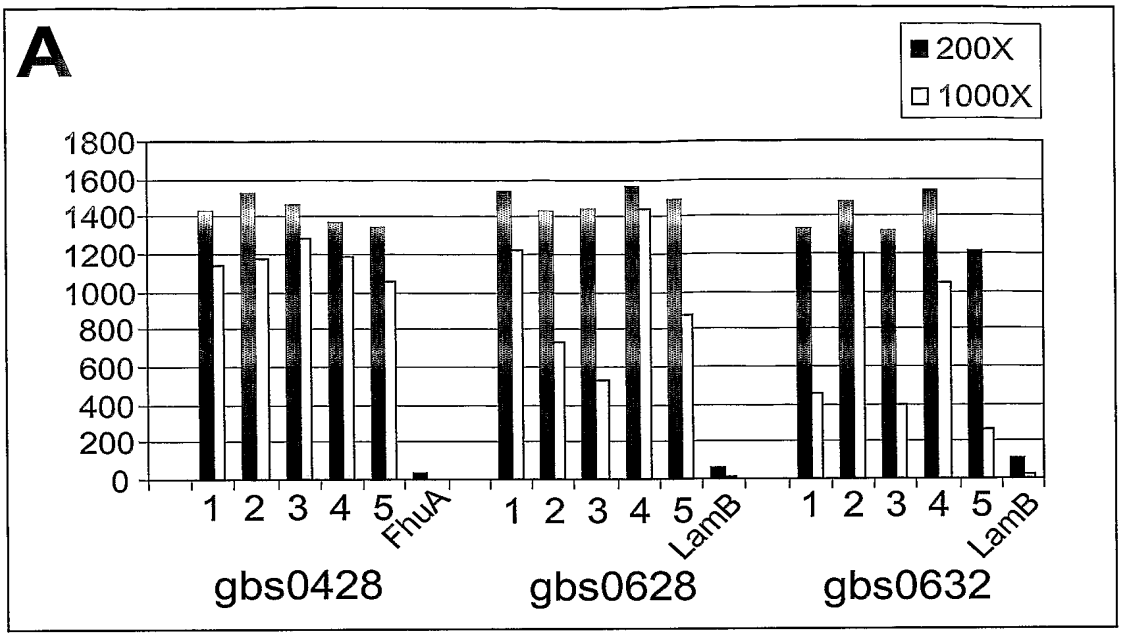

B
gbs0061
1 2 3 4 5 6 7 8 9 10 12 14 16 18 20 22 24
1500
600
26 28 30 32 34 36 38 40 42 44 46 - +
1500
600

Fig. 4


A
200X
1000X
1800
1600
1400
1200
1000
800
600
400
200
0
1 2 3 4 5 FhuA
1 2 3 4 5 LamB
1 2 3 4 5 LamB
gbs0428
gbs0628
gbs0632

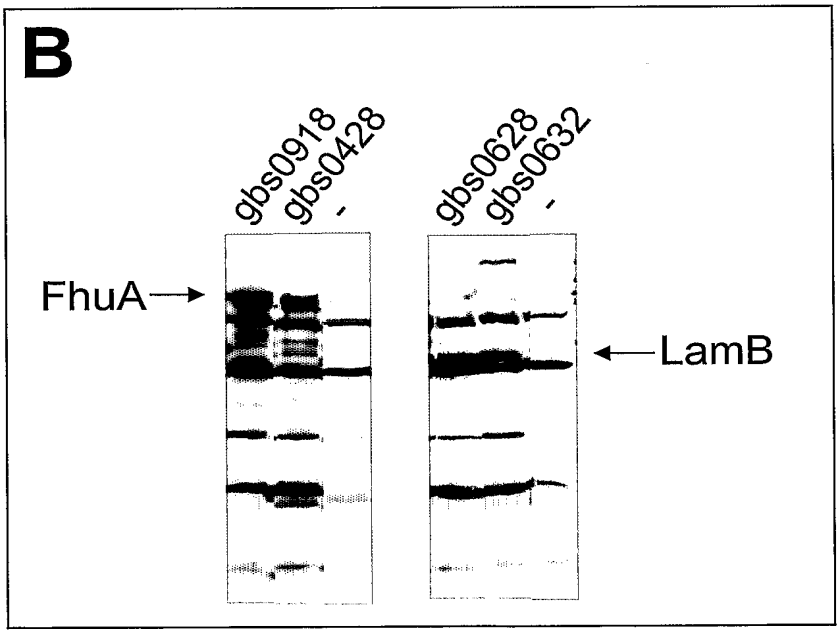

B
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FhuA
LamB

Fig. 5

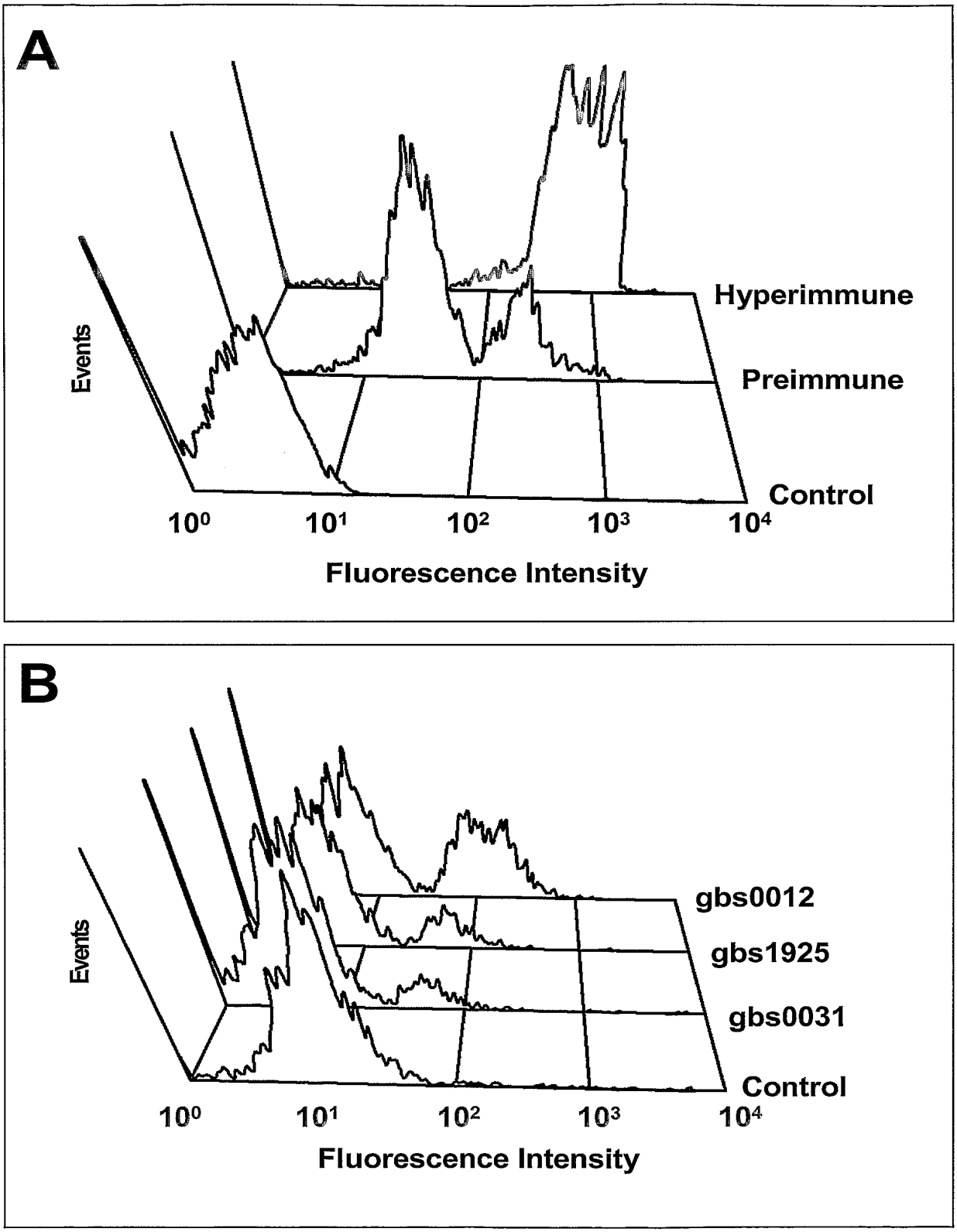

A
Events
Hyperimmune
Preimmune
Control
10⁰
10¹
10²
10³
10⁴
Fluorescence Intensity
B
Events
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gbs0031
Control
10⁰
10¹
10²
10³
10⁴
Fluorescence Intensity

Fig. 6

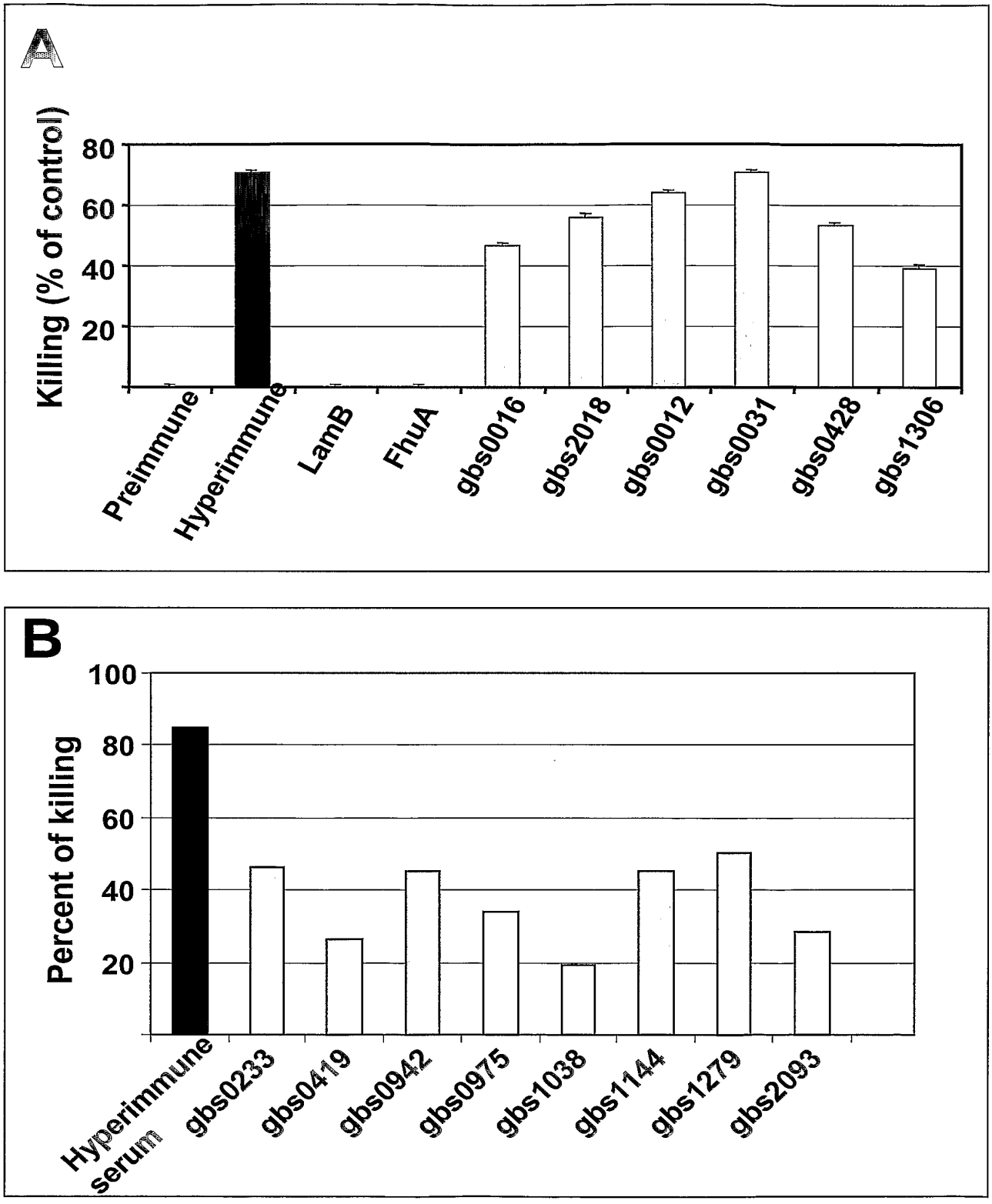

A
Killing (% of control)
80
60
40
20
Preimmune
Hyperimmune
LamB
FhuA
gbs0016
gbs2018
gbs0012
gbs0031
gbs0428
gbs1306
B
Percent of killing
100
80
60
40
20
Hyperimmune serum
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gbs0942
gbs0975
gbs1038
gbs1144
gbs1279
gbs2093

Fig. 7

SEQUENCE LISTING

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SeqID 197
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SeqID 198
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SeqID 207
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SeqID 209
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SeqID 210
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SeqID 212
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SeqID 213
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SeqID 218

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SeqID 219
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SeqID 220
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SeqID 221
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SeqID 222
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SeqID 223
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SeqID 224
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SeqID 225
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SeqID 226
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SeqID 227
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SeqID 228
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SeqID 229
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SeqID 230
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SeqID 231
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SeqID 232
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SeqID 233
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SeqID 234

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SeqID 235
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GVAQ

SeqID 236
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SeqID 237
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TLHGDGCLMEGVSQEAASLAGHLKLGKLVLLYDSNDISLDGPTSQSFTEDVKGRFESYGWQHILVKDGNDLEAIAAA
IEAAKAETDKPTIIEVKTIIGFGAEKQGTSSVHGAPLGAEGITFAKKAYGWEYPDFTVPAEVVARFASDLQARGAKA
EEAWNDLFAKYEVEYPELAAEYKEAFAGQAETVELKAHDLGSSVASRVSSQQAIQQLSTQLPNLWGGSADLSASNNT
MVAAETDFQASNYAGRNIWFGVREFAMAAAMNGIALHGGTRVYGGTFFVFSNYLLPAVRMAALQNLPTVYVMTHDSI
AVGEDGPTHEPIEQLASVRSMPNLNVIRPADGNETNAAWQRAVSETDRPTMLVLTRQNLPVLEGTSELAQEGVNKGA
YILSEAKGELDGIIIATGSEVKLALDTQDKLESEGIHVRVVSMPAQNIFDEQEASYQEQVLPSAVTKRLAIEAGSSF
GWGKYVGLNGLTLTIDTWGASAPGNRIFEEYGFTVENAVSLYKEL

SeqID 238
MTLQDQIIKELGVKPVINPSQEIRRSVEFLKDYLLKHSFLKTYVLGISGGQDSTLAGRLAQLAVEELRADTGENYQF
IAIRLPYGIQADEEDAQKALDFIKPDIALTINIKEAVDGQVRALNAAGVEITDFNKGNIKARQRMISQYAVAGQYAG
AVIGTDHAAENITGFFTKFGDGGADLLPLFRLNKSQGKQLLAELGADKALYEKIPTADLEENKPGIADEIALGVTYQ
EIDAYLEGKVVSDKSRGIIENWWYKGQHKRHLPITIFDDFWK

SeqID 239
IKKESVIKLLKYAFGIIMGFIILAIVIGGLLFAYYVSRSPKLTDQALKSVNSSLVYDGNNKLIADLGSEKRESVSAD
SIPLNLVNAITSIEDKRFFKHRGVDIYRILGAAWHNLVSSNTQGGSTLDQQLIKLAYFSTNKSDQTLKRKSQEVWLA
LQMERKYTKEEILTFYINKVYMGNGNYGMRTTAKSYFGKDLKELSIAQLALLAGIPQAPTQYDPYKNPESAQTRRNT
VLQQMYQDKNISKKEYDQAVATPVTDGLKELKQKSTYPKYMDNYLKQVISEVKQKTGKDIFTAGLKVYTNINTDAQK
QLYDIYNSDTYIAYPNNELQIASTIMDATNGKVIAQLGGRHQNENISFGTNQSVLTDRDWGSTMKPISAYAPAIDSG
VYNSTGQSLNDSVYYWPGTSTQLYDWDRQYMGWMSMQTAIQQSRNVPAVRALEAAGLDEAKSFLEKLGIYYPEMNYS
NAISSNNSSSDAKYGASSEKMAAAYSAFANGGTYYKPQYVNKIEFSDGTNDTYAASGSRAMKETTAYMMTDMLKTVL
TFGTGTKAAIPGVAQAGKTGTSNYTEDELAKIEATTGIYNSAVGTMAPDENFVGYTSKYTMAIWTGYKNRLTPLYGS
QLDIATEVYRAMMSYLTGGYSADWTMPEGLYRSGSYLYINGTTTTGTYSSSVYKNIYQNSGQSSQSSSSTSSEKQKE
DKNTANDANSSSPQVETPNNGNATTPNNSNQTVPGTGHGNGNGNNNTVPNGN

SeqID 240
MLDLKRIRTDFDVVAKKLATRGVDQETLTTLKELDIKRRELLIKAEEAKAQRNVASAAIAQAKRNKENADEQIAAMQ
TLSADIKAIDAELADVDANLQSMVTVLPNTPADDVPLGADEDENVEVRRWGTPREFDFETKAHWDLGESLGILDWER
GAKVTGSRFLFYKGLGARLERAIYSFMLDEHAKEGYTEVIPPYMVNHDSMFGTGQYPKFKEDTFELADSPFVLIPTA
EVPLTNYYRDEIIDGKELPIYFTAMSPSFRSEAGSAGRDTRGLIRLHQFHKVEMVKFAKPEESYQELEKMTANAENI
LQKLNLPYRVITLCTGDMGFSAAKTYDLEVWIPAQNTYREISSCSNTEDFQARRAQIRYRDEVDGKVRLLHTLNGSG
LAVGRTVAAILENYQNEDGSVTIPEVLRPYMGNIDIIKPN

SeqID 241
VTISNQELTLTPLRGKSGKAYIGTYPNGERVFVKYNTTPILPALAKEQIAPQLLWARRTSNGDMMSAQEWLDGRTLT
KEDMGSKQIIHILLRLHKSRPLVNQLLQLGYKIENPYDLLMDWEKQTPIQIRENTYLQSIVTELKRSLPEFRTEVAT
IVHGDIKHSNWVITTSGLIYLVDWDSVRLTDRMYDVAYILSHYIPQKHWKDWLSYYGYKDNEKVWSKIIWYGQFSYL
SQIIKCFDKRDMEHVNQEIYELRKFRELIKKHNAS

SeqID 242
MKISQYNKWSIRRLKVGAASVMIASGSIVALGQSHIVSADEMSQPKTTITAPTANTSTNVESSTDKALSKVTTMETS
SEMPKMQNMAKVEKTSDKPMMVATSVRKMMATPTPVAMTKTTSVDEVKKSTDTAFKQTVDVPAHYVNAAKGNGPFLA
GVNQTIPYEAFGGDGMLTRLILKSSEGAKWSDNGVDKNSPLLPLKGLTKGKYFYQVSLNGNTTGKEGQALLDQIKAN
DKHSYQATIRVYGAKDGKVDLKNMISQKMVTINIPHITTDMEVKNSLKMAFKEKVDVPAKYVSAAKAKGPFLAGVNE
TIPYEAFGGDGMLTRLILKASEGAKWSDNGVDKNSPLLPLKDLTKGKYFYQVSLNGNTAGKKGQALLDQIKANGSHT
YQATITIYGTKDGKVDMNTILGQKTVMIHINVAKKDMNSTSMMMKKDKMTMPMKKEMTSSKINTGMMMSNNKMSANM
QMSSQAKSNDKAGKKMSMMSKNLPNTGETKQQNVGVLGMLSLAFATGLTALGLKKSKQR

SeqID 243
MTHITTFDYSKVLGQFVGEHELDYLQPQVSAADAFLRQGTGPGSDFLGWMDLPENYDKEEFSRIQKAAEKIKSDSEVL
VVIGIGGSYLGAKAAIDFLNNHFANLQTAEERKAPQILYAGNSISSTYLADLVEYVQDKEFSVNVISKSGTTTEPAI
AFRVFKELLVKKYGQEEANKRIYATTDKVKGAVKVEADANNWETFVVPDNVGGRFSVLTAVGLLPIAASGADITALM
EGANAARKDLSSDKISENIAYQYAAVRNVLYRKGYITEILANYEPSLQYFGEWWKQLAGESEGKDQKGIYPTSANFS
TDLHSLGQFIQEGYRNLFETVVRVEKPRKNVTIPELTEDLDGLGYLQGKDVDFVNKKATDGVLLAHTDGGVPNMFVT
LPTQDAYTLGYTIYFFELAIGLSGYLNSVNPFDQPGVEAYKRNMFALLGKPGFEELSAELNARL

SeqID 244
MKEKQTAGRRQLEEFAPEFARYNDDILFGEVWAKEDHLTDKTRSIITISALISGGNLEQLEHHLQFAKQNGVTKEEI
ADIITHLAFYVGWPKAWSAFNKAKEIWI

SeqID 245
AWRALEDAQKSGKVKSIGVSNFLEKDLENILKNGHVKPAVNQILAHIGNTPFDLIDYCQSKGIQVEAYSPIAHGQAL
KSDGIQKMAEKYGVSVAQLCIQYLLQLNLIVLPKASSKEHLQSNLDFDFVISDEDMSILKSLMFDDYGEFSNFPVFS
EK

SeqID 246
MFRRSKNNSYDTSQTKQRFSIKKFKFGAASVLIGLSFLGGVTQGNLNIFEESIVAASTIPGSAATLNTSITKNIQNG
NAYIDLYDVKNGLIDPQNLIVLNPSSYSANYYIKQGAKYYSNPSEITTTGSATITFNILDETGNPHKKADGQIDIVS
VNLTIYDSTALRNRIDEVINNANDPKWSDGSRDEVLTGLEKIKKDIDNNPKTQIDIDNKINEVNEIEKLLVVSLPDK
IKYSPEAKHRTVEQHAELDAKDSIANTDELPSNSTYNWKNGHKPDTSTSGEKDGIVEVHYPDGTVDDVNVKVTVTSK
KTDNTAPTLTVTPEQQTVKVDEDITFTVTAEDENEVELGLDDLKAKYENDIIGARVKIKYLTKEPNKKVMEVTIMKA
TLADKGAITFTAKDKAGNQAEPKTVTINVLPPDKIKYSPEAKHRTVEQHAELDAKDSIANTDELPSNSTYNWKNGHK
PDTSTSGEKDGIVEVHYPDGTVDDVNVKVTVTSKKTDNTAPTLTVTPEQQTVKVDEDITFTVTAEDENEVELGLDDL
KAKYENDIIGARVKIKYLTKEPNKKVMEVTIMKATLADKGAITFTAKDKAGNQAEPKTVTINVLPPDKIKYSPEAKH
RTVEQHAELDAKDSIANTDELPSNSTYNWKNGHKPDTSTPGEKNAVVVVTYPDKSTDEVPVKVTVVDPRTDAEKNDP
AGKDQTVKVGEQPDPTKSLEAVPAGSTVAYKEPVDTKTPGEKNAIVVVTYPDKSTDEVPVKVTVVDPRTDAEKNDPA
GKDQTVKVGEQPDPTKSLEAVPAGSTVAYKEPVDTKTPGEKNAIVVVTYPDKSTDEVPVKVTVVDPRTDAEKNDPAG
KDQTVKVGEQPDPTKSLEAVPAGSTVAYKEPVDTKTPGEKNAVVVVTYPDKSTDEVPVKVTVVDPRTDAEKNDPAGK
DQTVKVGEQPDPTKSLEAVPAGSTVAYKEPVDTKTPGEKNAVVVVTYPDKSTDEVPVKVTVVDPRTDAEKNDPAGGE
TTVPQGTPISDEEITGLVKIPEGSNGVPKVVGDRPNTDVPGDYKVTVEVTYPDGTKDTVAVTVHVTPKPVPDKDKYD
PTGKSQQVNGKGNKLPATGESATPFFNVAALTIISSVGLLSVSKKKED

SeqID 247
MQILEDYDGRALPKLETDRLILRQRTVGDVPAMFDYVCLEEVAYPAGLSPIASLEDEYDYFENRYYQNLEKAKLPSG
YGITVKGSDRIIGSCAFNHRREDDVFEIGYLLHPDYWGHGYMTEAVAALIEVGFTLLNLHKIEIRCYDYNKQSQRVA
EKLGFTLEATIRDRKDNQGNRCVNLIYGLLRSEWE

SeqID 248
MSKELSPKYNPAEVEEGRYQTWLDQDVFKPSGDTEAKPYSIVIPPPNVTGKLHLGHAWDTTLQDIIIRQKRMQGFDT
LWLPGMDHAGIATQAKVEERLREQGISRYDLGREKFLDKVWEWKDEYAATIKSQWGKMGLSVDYSRERFTLDEGLSK
AVRKVFVDLYNKGWIYRGEFIINWDPAARTALSDIEVIHKDVEGAFYHMNYMLEDGSRALEVATTRPETMFGDVAVA
VNPEDPRYKDLIGQNVILPIINKPIPIIADEHADPEFGTGVVKITPAHDPNDFAVGQRHNLPQVNVMNDDGTMNELA
DEFNGMDRFEARKAVVAKLESLGNLVKIEKMTHSVGHSERTGVVVEPRLSTQWFVKMDQLAKNAIANQDTEDKVEFY
PPRFNDTFMSWMENVHDWVISRQLWWGHQIPAWYNVNGEMYVGEDAPEGDGWTQDEDVLDTWFSSALWPFSTMGWPD
TEAADFKRYFPTSTLVTGYDIIFFWVSRMIFQSLEFTGRQPFSNVLIHGLIRDEEGRKMSKSLGNGIDPMDVIEKYG
ADALRWFLSNGSAPGQDVRFSYEKMDASWNFINKIWNISRYILMNNEGLTLDQARENVEKVVNSQVGNVTDRWILHN
LNETVGKVTESFDKFEFGVAGHILYNFIWEEFANWYVELTKEVLYSDNEDEKVVTRSVLLYTLDQILRLLHPIMPFV
TEEIFGQYAEGSIVLASYPQVNATFENQTAHKGVESLKDLIRSVRNSRAEVNVAPSKPITILVKTSDSELESFFKDN
SNYIKRFTNPETLEISSAITAPELAMTSIITGAEIFLPLADLLNVEEELARLEKELAKWQKELNMVGKKLSNERFVA
NAKPEVVQKEKDKQTDYQTKYDATIARIEEMKKLNND

SeqID 249

MVEPIISIQGLHKSFGKNEVLKGIDLDIHQGEVVVIIGPSGSGKSTFLRTMNLLEVPTKGTVTFEGIDITDKKNDIF
KMREKMGMVFQQFNLFPNMTVLENITLSPIKTKGLSKLDAQTKAYELLEKVGLKEKANAYPASLSGGQQQRIAIARG
LAMNPDVLLFDEPTSALDPEMVGEVLTVMQDLAKSGMTMVIVTHEMGFAREVADRVIFMDAGIIVEQGTPKEVFEQT
KEIRTRDFLSKVL

SeqID 250
MNYKEIYQEWLENDSLGKDIKSDLEAIKGDESEIQDRFYKTLEFGTAGLRGKLGAGTNRMNTYMVGKAAQALANTII
DHGPEAIARGIAVSYDVRYQSKEFAELTCSIMAANGIKSYIYKGIRPTPMCSYAIRALGCVSGVMVTASHNPQAYNG
YKAYWKEGSQILDDIADQIANHMDAITDYQQIRQIPFEEALASGLASYIDESIEEAYKKEVLGLTINDTNIDKSVRV
VYTPLNGVGNLPVREVLRRRGFENVYVVPEQEMPDPDFTTVGYPNPEVPKAFAYSESLGKSVDADILLATDPDCDRV
ALEVKDSKGEYIFLNGNKIGALLSYYIFSQRCALGNLPHHPVLVKSIVTGDLSKVIADKYNIETVETLTGFKNICGK
ANEYDISKDKTYLFGYEESIGFCYGTFVRDKDAVSASMMVVEMTAYYKERGQTLLDVLQTIYDEFGYYNERQFSLEL
EGAEGQERISRIMEDFRQDPILQVGEMRLENSIDFKDGYKDFPKQNCLKYYFNEGSWYALRPSGTEPKIKCYLYTIG
CTEADSLSKLNAIESACRAKMNSTK

SeqID 251
MYREITAVEHDRFVSESNQTNLLQSSNWPKVKDNWGSQLLGFFDGETQIASASILIKSLPLGFSMLYIPRGPIMDYS
NLDIVTKVLKDLKAFGKKQRALFIKCDPLIYLKMVNAKDFENSPDEKEGLIAIDHLQRAGADWTGRTTDLAHTIQPR
FQANLYANQFGLDKMSKKTRQAIRTSKNKGVDIQFGSHELLEDFAELMKKTEDRKGINLRGIDYYQKLLDTYPNNSY
ITMASLDVAKRLEKIEKECQIAQSERIKSLELNREKKVKQHQGTIDRLNKEIDFLKEAQKAYDRDIIPLAATLTLEF
GNTSENIYAGMDDYFKSYSAPIYTWFETAQRAFERGNIWQNMGGIENDLSGGLYHFKSKFEPIIEEFIGEFNIPVNR
LLYKASNYVYALRKKRNS

SeqID 252
MACTTILVGKKASYDGSTMIARTEDSVNGDFTPKKLKVMTSKDQPRHYKSVLSNFEVDLPDNPLPYTSVPDALGKDG
IWGEAGINSKNVAMSATETITTNSRVLGADPLVSDGIGEEDILTLVLPYIQSAREGVERLGAILEKYGTYESNGIAF
SDTEEIWWLETIGGHHWIARRVPDDVYVTNPNQLGIDHFEFNNCDDYMCSSDLKEFIEQYHLDLTYSNEHFNPRYAF
GSQRDKDRHYNTPRSWAMQRFLNPEIEQDPRSLFIPWCQKPYRKITVEDIKYVLSDHYQDSVYDPYGPEGDAVSRRA
FRSVGINRTSQTSILQLRPNKSLETTGVQWLSYGSMPFATMVPLFTQVETVPNYFSNTTKDASTDNFYWTNRLIAAL
ADPHFYQHEADIESYIERTMAQGHAHINGVDREVAENKEIDFQQKNQEMSDYIQKESQELLNRILFDASNLMTNRFS
MGD

SeqID 253
MRKKFLLLMSFVAMFAAWQLVQVKQVWADSKLKVVTTFYPVYEFTKNVVGDKADVSMLIKAGTEPHDFEPSTKNIAA
IQDSNAFVYMDDNMETWAPKVAKSVKSKKVTTIKGTGDMLLTKGVEEEGEEHEGHGHEGHHHELDPHVWLSPERAIS
VVENIRNKFVKAYPKDAASFNKNADAYIAKLKELDKEYKNGLSNAKQKSFVTQHAAFGYMALDYGLNQVPIAGLTPD
AEPSSKRLGELAKYIKKYNINYIYFEENASNKVAKTLADEVGVKTAVLSPLEGLSKKEMAAGEDYFSVMRRNLKVLK
KTTDVAGKEVAPEEDKTKTVETGYFKTKDVKDRKLTDYSGNWQSVYPLLQDGTLDPVWDYKAKSKKDMTAAEYKKYY
TAGYKTDVESIKIDGKKHQMTFVRNGKSQTFTYKYAGYKILTYKKGNRGVRYLFEAKEKDAGQFKYIQFSDHGIKPN
KAEHFHIFWGSESQEKLFEEMENWPTYFPAKMSGREVAQDLMSH

SeqID 254
MKLSKKLLFSAAVLTMVAGSTVEPVAQFATGMSIVRAAEVSQERPAKTTVNIYKLQADSYKSEITSNGGIENKDGEV
ISNYAKLGDNVKGLQGVQFKRYKVKTDISVDELKKLTTVEAADAKVGTILEEGVSLPQKTNAQGLVVDALDSKSNVR
YLYVEDLKNSPSNITKAYAVPFVLELPVANSTGTGFLSEINIYPKNVVTDEPKTDKDVKKLGQDDAGYTIGEEFKWF
LKSTIPANLGDYEKFEITDKFADGLTYKSVGKIKIGSKTLNRDEHYTIDEPTVDNQNTLKITFKPEKFKEIAELLKG
MTLVKNQDALDKATANTDDAAFLEIPVASTINEKAVLGKAIENTFELQYDHTPDKADNPKPSNPPRKPEVHTGGKRF
VKKDSTETQTLGGAEFDLLASDGTAVKWTDALIKANTNKNYIAGEAVTGQPIKLKSHTDGTFEIKGLAYAVDANAEG
TAVTYKLKETKAPEGYVIPDKEIEFTVSQTSYNTKPTDITVDSADATPDTIKNNKRPSIPNTGGIGTAIFVAIGAAV
MAFAVKGMKRRTKDN

SeqID 255
MKKRQKIWRGLSVTLLILSQIPFGILVQGETQDTNQALGKVIVKKTGDNATPLGKATFVLKNDNDKSETSHETVEGS
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VNVEGSKVGEQYKALNPINGKDGRREIAEGWLSKKITGVNDLDKNKYKIELTVEGKTTVETKELNQPLDVVVLLDNS
NSMNNERANNSQRALKAGEAVEKLIDKITSNKDNRVALVTYASTIFDGTEATVSKGVADQNGKALNDSVSWDYHKTT
FTATTHNYSYLNLTNDANEVNILKSRIPKEAEHINGDRTLYQFGATFTQKALMKANEILETQSSNARKKLIFHVTDG
VPTMSYAINFNPYISTSYQNQFNSFLNKIPDRSGILQEDFIINGDDYQIVKGDGESFKLFSDRKVPVTGGTTQAAYR
VPQNQLSVMSNEGYAINSGYIYLYWRDYNWVYPFDPKTKKVSATKQIKTHGEPTTLYFNGNIRPKGYDIFTVGIGVN
GDPGATPLEAEKFMQSISSKTENYTNVDDTNKIYDELNKYFKTIVEEKHSIVDGNVTDPMGEMIEFQLKNGQSFTHD
DYVLVGNDGSQLKNGVALGGPNSDGGILKDVTVTYDKTSQTIKINHLNLGSGQKVVLTYDVRLKDNYISNKFYNTNN
RTTLSPKSEKEPNTIRDFPIPKIRDVREFPVLTISNQKKMGEVEFIKVNKDKHSESLLGAKFQLQIEKDFSGYKQFV
PEGSDVTTKNDGKIYFKALQDGNYKLYEISSPDGYIEVKTKPVVTFTIQNGEVTNLKADPNANKNQIGYLEGNGKHL
ITNTPKRPPGVFPKTGGIGTIVYILVGSTFMILTICSFRRKQL

SeqID 256
GAKKAGWTEYARMLEVREQVDHVMIPKINQDLPIYAGPEEDNLQRGVGHLEGISLPIGGASTHAVLSGQRGMPAARL
FADLDKMKKGDYFYVTNLKETLAYQVDRIMVIEPSQLDAVSIEEDKDYVTLLTCTPYMGSLSTVMGDLSLTTRENQL
GSLSFWMFKAMRILLLKFLKLRKPKACRLMSLISLLVASNLALERDS

SeqID 257
MSDVVEKQTAKSFIMNVLNGLALGTVIVLIPGAILGELMKALLPMWSGFATLIAATAVATSMMGLVIGIMVGLNFKF
NPIQSASLGLAVMFAGGAATFLKGAIMLKGTGDIINMGITAALGVLLIQFLSDKTKSFTLIVIPTVTLLLVGGVGHV
LLPYVKMITTMIGQGIASLLGLQPVLMSILIAMIFCFLIVSPITTVGIALAISLSGIGSGAANLGICAASFGLCMAG
WSVNSKGTALAHVLGSPKISMANVLAKPKIMLPMISSAAILGILGALFNIQGTPASAGFGISGLIGPINALNLAKGG
WSVMNMLLIIIIFVAAPIILNFIFNYLFIKVLKIIDPMDYKLDI

SeqID 258
MARPLVEQTADRLLHLILEREYPVGAKLPNEYELAEDLDVGRSTIREAVRSLATRNILEVRQGSGTYISSKKGVSED
PLGFSLIKDTDRLTSDLFELRLLLEPRIAELVAYRITDDQLQLLEKLVGDIEDAVHAGDPKHLLLDVEFHSMLAKYS
GNIAMDSLLPVINQSIHLINANYTNRQMKSDSLEAHREIIKAIREKNPVAAHDAMLMHIMSVRRSALKA

SeqID 259
MIKKNKVFLGVLLVLVVILGGGVLFYQSQFQKTTNQALAIAYKDAKVAKKDVIHQKIDKEFENFRGSYEIEFNTKSA
EYSYHVDAKTGQILERDMDNNGFSKSTSQSSSSSSQKSHKISQEEAKKIAFKDANIEESEVSNLKIKEEIENGKSVY
DIDFVDLKNKNEVDYQIDAETGKIIERSRDHMND

SeqID 260
LNRKKRYRLTVKKQNASIPRRLNLLFFIIVLLFTVLILRLEQMQIGQQSFYMKKLTALTSYTVKESKARGQIFDAKG
VVLVENDERPTVAFSRGNNISSQSIKELANKLSHYITLTEVASSDRAKRDYYLADKANYKKVVESLPDSKRYDKFGN
HLAESTVYANAVAAVPVSAINYSEDELKVVALFNQMNATPTFGSVKLSTGELSDDQIKKLDADKKELLGISVTSNWH
RRKKGTSLSDILGTISTEKAGLPREEVKKYLKKGYSLNDRVGTSYLEKQYEDDLQGIRQIRKVVVNKKGKVVSDNIT
QEGKSGRNLKLTIDLNYQNKVESILKQYYGSELSSGRASFSEGMYAVAIEPSTGKVLAMAGLKNDHGNLVDDSLGTI
AKNFTPGSVVKGATLSSGWENKVLRGNEVLYDQEIANIRSWFTRGLTPISAAQALEYSSNTYMVQVALRLMGQDYNT
GDALTDRGYQEAMAKLRKTYGEYGLGVSTGLDLPESEGYVPGKYSLGTTLMESFGQYDAYTPMQLGQYISTIANNGN
RLAPHVVSDIYEGNDSNKFAQLVRSITPKTLNKIAISDQELAIIQEGFYNVVNSGSGYATGTSMRGNVTTISGKTGT
AETFAKNVNGQTVSTYNLNAIAYDTNRKIAVAVMYPHVTTDTTKSHQLVARDMIDQYISQFTGQ

SeqID 261
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VFRTSIAWSRIFPNGDDSAPNEAGLQFYDNLFDELLKYNIEPLVTLSHYETPLHLAKTYNGWADRRLIAFFEKFAQT
VMERYKDKVKYWLTFNEVNSILHMPFTSGAIMTDKSQLSPQELYQAIHHELVASARVTKLGRSINPNFKIGCMILAM
PAYPMTSDPRDVLAARQFEQHNLLFSDIHVRGKYPTYIQSYFKNNGIKIKFEEGDEEVLAQNTVDFLSFSYYMSVTQ
AYDFENYQSGQGNILGGLTNPHLTTSEWGWQIDPIGLRLVLNQYYERYQIPLFIVENGLGAKDQLIETLDGDYTVED
DYRIDYMNQHLVQVAKAIEDGVEIMGYTSWGCIDCVSMSTAQLSKRYGLIYVDRNDDGTGSLQRYKKKSFGWYQKVI
KTNGQSLFEHHNR

SeqID 262
MATFQIKEKMFSLGGKFTITDQTGLPCYHVEGSLFPLPKTFKVFDEEGHLISQIEKKVLSFLPKFNVTLANGNHFTI
KKDFSFLKPHYTIEDLDMEVKGNFWDIDFQLLKDNQVIANISQQWFRMTSTYQVEVYNETYNDLTISLVIAIDYVKE
LEKNASN

SeqID 263
MKKITTLILASSLLLVATTSVKADDNFEMPTRYVKMSEKSKAFYQRLQEKQRKAHTTVKTFNNSEIRHQLPLKQEKA
RNDIYNLGILISQESKGFIQRIDNAYSLENVSDIVNEAQALYKRNYDLFEKIKSTRDKVQVLLASHQDNTDLKNFYA
ELDDMYEHVYLNESRVEAINRNIQKYN

SeqID 264
MKLKKFFEDLLAKLEYRPIQVFMRHFQSAEMDLSAIAVAYYLLVTAFPLLVIAANIFPYFHINVSDLLSLMQKNLPK
NIYEPASRLAVDAFSKPSTGILGFASLTAFWTMSKSLTSLQKAINKAYGVDQHRDFVISRLVGVGTGLIILFLLTFV
LIFSTFSKPVLQIIVNMYDLGDTLTAWLLNLAQPVTFLTIFLGIGILYFILPNARIRKVRYVIPGTLFSTFVIGFFS
NLISQYVLNRVEKMVDIKTFGSVVIFILMLWFIFLAHIMILGAILNASVQEIATGKIESRRGDIMSLIQKSKEEK

SeqID 265
MKLLYTDINHDMTEILVNQAAHAAEAGWRIFYIAPNSLSFEKERAVLENLPQEASFAITITRFAQLARYFTLNQPNQ
KESLNDIGLAMIFYRALASFEDGQLKVFGRLKQDASFISQLVDLYKELQTANLSILDLKYLHSPEKFEDLLAIFLVV
SDLLREGEYDNQSKIAFFTEQVRSGQLDVDLKNTILIVDGFTRFSAEEEALIKSLSSRCQEIIIGAYASQKAYKANF
TNGNIYSAGVDFLRYLATTFQTKPEFILSKWESKSGFEMISKNIEGKHDFTNSSHILDDTAKDCITIWECINQKDEV
EHVARAIRQKLYQGYRYKDILVLLGDVDSYKLQLSKIFEQYDIPYYFGKAETMAAHPLVHFMDSLSRIKRYRFRAED
VLNLFKTGIYGEISQDDLDYFEAYISYADIKGPKKFFTDFVVGAKKFDLGRLNTIRQSLLAPLESFVKTKKQDGIKT

LNQFMFFLTQVGLSDNLSRLVGQMSENEQEKHQEVWKTFTDILEQFQTIFGQEKLNLDEFLSLLNSGMMQAEYRMVP
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LFNAAKQALVLTIPQLLNESEDQMSPYLIELRDIGVPFNHKGRQSLKEEADNIGNYKALLSRVVDLYRSAIDKEMTK
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EQDSIHPDMRHHGTYLHRVFEILMKNQGIESFEEKLNSAINKTNQEDVFKSLYSEDAESRYSLEILEDIARATATIL
RQDSQMTVESEEERFELMIDNTIKINGIIDRIDRLSDGSLGVVDYKSSAQKFDIQKFYNGLSPQLVTYIDAISRDKE
VEQKPPIFGAMYLHMQEPKQDLSKIKNLDDLVTKNHQALTYKGLFSEAEKEFLANGKYHLKDSLYSEAEIAILQAHN
QLLYKKASETIKSGKFLINPYTEDAKTVDGDQFKSITGFEADRHMARARALYKLPAKEKRQGFLTLMQQEEENDDL

SeqID 266
MSETKVMALREAINVAMSEEMRKDEKVFLMGEDVGVYGGDFGTSVGMLEEFGAKRVRDTPISEAAIAGSAIGAAQTG
LRPIVDLTFMDFVTIAMDAIVNQGAKTNYMFGGGLSTPVTFRVASGSGIGSAAQHSQSLEAWLTHIPGLKVVAPGTV
NESKALLKSSILDNNPVIFLEPKALYGKKEEVNMDPDFYIPLGKGDIKREGTDLTIVSYGRMLERVMQAAEEVAEEG
INVEVVDPRTLIPLDKELIIDSVKKTGKLILVNDAYKTGGFTGEIATMVAESEAFDYLDHPIVRLASEDVPVPYSRV
LEQGILPDVAKIKDAIYKVVNKGK

SeqID 267
MAFDVIMPKLGVDMQEGEILEWKKNEGDTVNEGDVLLEIMSDKTNMEIEAEDTGVLLKIVHQAGDVVPVTEVIAYIG
EEGEEVGTSSPSADATITAEDGQSVSGPAAPSQETVAAATPKEELAADEYDIVVVGGGPAGYYAAIRGAQLGGKIAI
VEKTEFGGTCLNVGCIPTKTYLKNAEILDGLKVAAGRGINLASTNYAIDMDKTVAFKNSVVKTLTGGVRGLLKANKV
EIFNGLGQVNPDKSVVIGDKVIKGRNVVLATGSKVSRINIPGIESPLVLTSDDILDLREIPKSLAVMGGGVVGIELG
LVWASYGVDVTVIEMADRIIPAMDKEVSLELQKILAKKGMKIKTSVGVSEIVEANNQLTLKLNNGEEVVADKALLSI
GRVPQMNGLENLEPELEMERGRIKVNAYQETSIPGIYAPGDVNGTRMLAHAAYRMGEVAAENALGGNKRKAHLDFTP
AAVYTHPEVAMVGMTEEQAREQYGDILVGKNSFTGNGRAIASNEAHGFVKVIAEPKYKEILGVHIIGPAAAELINEA
STIMENELTVYDVAQSIHGHPTFSEVMYEAFLDVLGEAIHNPPKRK

SeqID 268
MGKYFGTDGVRGEANVELTPELAFKLGRFGGYVLSQHETDRPRVFVARDTRISGEMLESALIAGLLSVGIEVYKLGV
LATPGVSYLVRTEKASAGVMISASHNPALDNGIKFFGSDGFKLDDDRELEIEALLDAKEDTLPRPSAQGLGTLVDYP
EGLRKYEKFMESTGIDLEGMKVALDTANGAATASARNIFLDLNADISVIGDQPDGLNINDGVGSTHPEQLQSLVREN
GSDIGLAFDGDSDRLIAVDENGEIVDGDKIMFIIGKYLSDKGQLAQNTIVTTVMSNLGFHKALDREGIHKAITAVGD
RYVVEEMRKSGYNLGGEQSGHVIIMDYNTTGDGQLTAIQLTKVMKETGKKLSELASEVTIYPQKLVNIRVENNMKDK
AMEVPAIAEIIAKMEEEMDGNGRILVRPSGTEPLLRVMAEAPTNEAVDYYVDTIADVVRTEIGLD

SeqID 269
MKGQKIIALAGLVLSCHFALTACHTQEHKNSHHIKTKQVAKKKANKKKVSVKESHKKRKGVAGVDFPTDDGFLLTKD
SKILSHPDSGIVVAHGNHSHFIFYSDLKGSKFSYLIPNANAKTNKKQAVRNFKAGAVAVNTLNDGYVFNPADIVSED
ANGYVVRHGDHFHYIPKASLSQQKQVQASRAVSRLGNQNNSHYRVNSSKIAGLHHPTSDGFLFNGQGIKGTTPTGIL
VEHHNHLHFISFADLRKGGWGSIADRYQPQKKVDSKKQSPSSKKPRTENTLPKDIKDKLAYLARELHLDISRIRVLK
TLNGEIGFEYPHDDHTHVIMAKDIDLSKPIPNPHHDDEDHHKGHHHDESDHKHEEHEHTKSNKLSDEDQKKLIYLAE
KLGLNPNQIEVLTSEDGSIIFKYPHDDHSHTIASKDIEIGKPIPDGHHDHSHAKDKVGMATLKQIGFDDEIIQDILH
ADAPTPFPSNETNPEKMRQWLATVTKINIGQRTNPFQRFGLSLMPNIEVLGIGFTPINDMTPVLQFKKLKQLWMTNT
GITDYSFLDKMPLLEGLDISQNGIKDLSFLTKYKQLSLIAAANNGITSLKPLAELPNLQFLVLSHNNISDLTPLSNL
TKLQELHLDHNNVKNLSALSGKKDLKVLDLSNNKSADLSTLKTTSLETLLLNETNTSNLSFLKQNPKVSNLTINNAK
LASLDGIEESDEIVKVEAEGNQIKSLVLKNKQGSLKFLNVTNNQLTSLEGVNNYTSLETLSVSKNKLESLDIKTPNK
TVTNLDFSHNNVPTSQLKLNEKNIPEAVAKNFPAVVEGSMVGNGSLAEKAAMASKEDKQVSDNTNHQKNTEKSAQAN
ADSKKENPKTHDEHHDHEETDHAHVGHHHH

SeqID 270
MNKRVKIVATLGPAVEFRGGKKFGESGYWGESLDVEASAEKIAQLIKEGANVFRFNFSHGDHAEQGARMATVRKAEE
IAGQKVGFLLDTKGPEIRTELFEDGSDFHSYTTGTKLRVATKQGIKSTPEVIALNVAGGLDIFDDVEVGKQILVDDG
KLGLTVFAKDKDTREFEVVVENDGLIGKQKGVNIPYTKIPFPALAERDNADIRFGLEQGLNFIAISFVRTAKDVNEV
RAICEETGNGHVKLFAKIENQQGIDNIDEIIEAADGIMIARGDMGIEVPFEMVPVYQKMIITKVNAAGKAVITATNM
LETMTDKPRATRSEVSDVFNAVIDGTDATMLSGESANGKYPVESVRTMATIDKNAQTLLNEYGRLDSSAFPRNNKTD
VIASAVKDATHSMDIKLVVTITETGNTARAISKFRPDADILAVTFDEKVQRSLMINWGVIPVLADKPASTDDMFEVA
ERVALEAGLVESGDNIVIVAGVPVGTGGTNTMRVRTVK

SeqID 271
MTATKQHKKVILVGDGAVGSSYAFALVNQGIAQELGIIEIPALFDKAVGDAEDLSHALAFTSPKKIYAATYADCADA
DLVVITAGAPQKPGETRLDLVGKNLAINKSIVTQVVESGFNGIFLVAANPVDVLTYSTWKFSGFPKERVIGSGTSLD
SARFRQALADKIGVDARSVHAYIMGEHGDSEFAVWSHANVAGVQLEQWLQENRDIDEQGLVDLFISVRDAAYSIINK
KGATYYGIAVALARITKAILDDENAVLPLSVYQEGQYGDVKDVFIGQPAIVGAHGIVRPVNIPLNDAELQKMQASAE
QLKDIIDEAWKNPEFQEASKN

SeqID 272

MQDKNLVDVNLTSEMKTSFIDYAMSVIVARALPDVRDGLKPVHRRILYGMNELGVTPDKPHKKSARITGDVMGKYHP
HGDSSIYEAMVRMAQWWSYRHMLVDGHGNFGSMDGDGAAAQRYTEARMSKIALEMLRDINKNTVDFQDNYDGSEREP
LVLPARFPNLLVNGATGIAVGMATNIPPHNLGESIDAVKLVMDNPDVTTRELMEVIPGPDFPTGALVMGRSGIHRAY
ETGKGSIVLRSRTEIETTSNGKERIVVTEFPYGVNKTKVHEHIVRLAQEKRIEGITAVRDESSREGVRFVIEVRRDA
SANVILNNLFKLTSLQTNFSFNMLAIEKGVPKILSLRQIIDNYIEHQKEVIVRRTQFDKAKAEARAHILEGLLVALD
HLDEVITIIRNSETDTIAQAELMSRFELSERQSQAILDMRLRRLTGLERDKIQSEYNDLLALIADLADILAKPERVV
TIIKEEMDEVKRKYADARRTELMIGEVLSLEDEDLIEEEDVLITLSNKGYIKRLAQDEFRAQKRGGRGIQGTGVNND
DFVRELVSTSTHDTVLFFTNLGRVYRLKAYEIPEYGRTAKGLPIVNLLKLDEGETIQTIINARKEDVANKYFFFTTQ
QGIVKRTSVSEFSNIRQNGLRAINLKENDELINVLLIDENEDVIIGTRTGYSVRFKVNAVRNMGRTATGVRGVNLRE
GDKVVGASRIVNGQEVLIITEKGYGKRTEASEYPTKGRGGKGIKTANITAKNGPLARLVTINGNEDIMVITDTGVII
RTNVANISQTGRSTMGVKVMRLDQEAKIVTVALVEQEIEDKSNIEDTKE

SeqID 273
MTIFDERELKERFTHENRVSFYEFVAKYDAQMVPVMKAKGYRCIHSMERTVVFTFGEFTIRRRRWQKGEHWVVPVDE
KLGLKKNVRYSLEFMYQIASLATMMPYEKVIKVVQMMYCIVITKPTVVKAVKISRELLKEKEAYRFFDEDIPVDKEP
VDMIYLEGDGVMVKAREEGLDNRNVDLSHFVVHTGSQKVGSNRFVLQNKKEFVSLDNRQTRQKILDYLYNHFYIAPN
TLLITNSDGGHGYTPYVFKEIAKALKVKQHEHFWDRYHVNEKIKSFFKLYPVELMTGAFQSIKQHDKEKLRTVLDTT
EALILMEEEMEGFNQFKRKLLNNFQYTKSAELRGFSRAGIGVMESQHRKITYRMKKRGMYWTIQGAETMSQLIVLSY
EGQLRDLFFGSWREDYQKYQELENLSAGKIKHEQNKINKRYDLQTLGRLRYGRHRNL

SeqID 274
MAYLSKLSDLDPSLMDADSEQIYIPKVLFEHNDFKGLTYKEILLYSFLLNRLREPLDFIQKGYDDNEDTYVHFKVED
LCELLNQSKTTVISLKKRLAQYGLIEEVKAGSHQPNRIYLTDKLVPYIKG

SeqID 275
MTDNRFAQLKENFEKGSPKRRVPTSRPIAAQKAPESYNKKGRYPFSLHQDVRYDKLEALVAYHGAKSASDYLERLIV
QEWEKMQRKLKNKEK

SeqID 276
MFSWLEALYYTLIQLAKVNRLNALFLVSVVGYLCYQGIKLVRKTIRNFFQLMKGFIGDRENIKKCIKNKKEALVHSW
KHRQDIDWKSTGKDKSKQLWNLMKRLATVAPSFLFLLLGNVLFRLIYQLPFVKQDRKRFDKEMKPLLYFKNYRSFVF
MGIGFSFIAFILTNYFVTVLRAAIRFLYFSIMTLRDNSQVVSFNVDSLLIQNLFNARVFVIAPILAVPIFLIGLVVA
WRSAWVNFEQYRDYNHNEEGDDRFATVKEIHQQYKKVPNKTETYPGEGGVPVLHETRKNLTGLTLKSQMLWQNRTFS
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RRRGYDVNVLSFQNMDWSMSYNPLALAIAAAKKGYYEMTQTRVNAVAEAIYRKTKPGSGNGNAKYWEDTSISLFNAI
AMALMDRANETVRNGETDAWDTVTVRNIAKFLTDLGSEEVFVNDFGEIVENPDKNQQVKKKSKITVYFDNLRKINQE
QFSKFRDMADLNFRSSDFASEETKGNVFSSMMSGINLFLQDNIAKLTSKNSIDLESVGFPRRLSIKFRSSSNVAMRN
EYTHKTAKVTITSQAVWGKTTKQVIHVDAATALIDGEGYLTYVIEPQLPDQFLVTIDFNHENNGGSAIRHKTFQFSA
EKVYKKRGNVITLDDYTKKPVLDHIKVTVLNKQDDNLLQKEDIDLIYSDNPKVIYLVTPPNRTEYNSIVSLFLDQLF
NANYELALSNGRKCVNRILHILDEFTNIPAIPHMDTKISIGLGQNILYYLWIQNLKQLVSEYGENTAETIRENCSLK
VYIKSTAPATNEYFSKELGTRTITRRRRSSNILDEANPNVSIENPRQELLTPTQLSKLQEGEAVILRGVKGRDNAGR
KITTDPIFLHEKTSLPYRYMFLQEEFDQSMALADIPVESGHRDLDLQDIAVGAQSTFNKIIDWRMALTDRMRTNGKI
PQLASRKQTIKALSQSQFTSPADLTQAVIAEVFDEEDDDDLFFVDDVM

SeqID 277
MNSNTKGHGFFRKSKAYGLVCAIALAGAFTLATSQVSADQVTTQATTQTVTQNQAETVTSTQLDKAVATAKKAAVAV
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GQAAVDARNKAKQQAQDDQKAKIDAENKAESQRVSQLNAQNKAKIDAENKDAQAKANATNAQLQKDYQAKLAEIKSV
EAYNAGVRQRNKDAQAKADATNAQLQKDYQAKLALYNQALKAKAEADKQSINNVAFDIKAQAKGVDNAEYGNSIMTA
KTKPDGSFEFNHDMIDGVKTIGYGKLTGKVNHHYVANKDGSVTAFVDSVTLYKYEYRNVAQNAAVNQNIVFRVLTKD
GRPIFEKAHNGNKTFAETLNKTLQLNLKYELKPHASSGNVEVFKIHDDWVHDTHGSALVSYVNNNDAVPNVVIPERP
TPPKPVKVTPEAEKPVPEKPVEPKLVTPTLKTYTPVKFIPREYKPEPITPETFTPEKFTPAQPKVKPHVSIPEKINY
SVSVHPVLVPAANPSKAVIDEAGQSVNGKTVLPNATLDYVAKQNFSQYKGIKASAEAIAKGFAFVDQPNEALAELTV
KSIKASNGDDVSSLLEMRHVLSKDTLDQKLQSLIKEAGISPVGEFYMWTAKDPQAFYKAYVQKGLDITYNLSFKIKA
NFTKGQIKNGVAQIDFGNGYTGNIVVNDVTVPEVHKDILDKEDGKSINNSTVKLGDEVTYKLEGWVVPANRGYDLFE
YKFVDQLQHTHDLYLRDKVVAKVDVTLKDGTVIKKGTNLGEYTETVYNKTTGHYELAFKKEFLAKVSRESEFGADDF
IVVKRIKAGDVYNTADLYVNGYKVKSEAVVTHTTEKSKPVEPQKATPKAPAKGLPSTGEASMTPLTAIGAIILSALG
LAGFKKRQK

SeqID 278
MKQIKIITGLTVATLSAVVGNVYAEDITPTAPVNEPQVSSETAKTPQVTESQVNSAKVTADQAISDVNNQQIVVDEA
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VNKQLAVTDTQTLLKKLVTEINNEKVSTSLENQAYFNQRDGSWAGYYGNYTFAATGCVPSSLAMVFTELARRQITPT
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RGYSNGNTYVYDPYNRANIGWYPVANLWNEQSRDAIDTSSVGVPFFKITTQKMAQLEAQKAQVQSSLNTAKNQLAKT
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VASQVKLADLKATLATVENNVKKAQATLTDAKAIVGQKQAKLLALQNAPKILADAQAKLVTAKNDLANKMAILDEAV
AKLKSLQAVQAEAQKQYHVVFEAYKAVRDAKEQAKLAESYNHIIARGGEAIPVVDETDKITGYVDGSQKAVANEVTL
ALTSNGAPLESPVNKENQNVTKSSQALPHTGEAGLSILSVLGVGLISTLGLTSLKKRRPH

SeqID 279
VRLTHVSLWMPSKRSWPLKLQLLRKGVIELEDELKTPYLDQYTDNLTAKVTKKSDDYQVYGRNKEVQSVIISLLRRT
KNNPILVGEAGVGKSAIVEGITLAILRGQVPEPLKGLTVRSLELSSLMSEDDEGFIAKFKKIIEEMVATRGHNLLFV
DEFHTIIGAGSQNGQALDAGNVIKPVLARGDIQLIGATTLDEFHEYIETDRALERRMQPVMVEEPTISQAITIIEQA
KVIYEKFHGIQISSDAVHQAIRLSVRYLTDRFLPDKAFDLIDEAATIASVEGKSKVTEKDIAQVLKDKTGIPVTTIL
KGDQERLEGFKERLMNRVKGQEDAIEAVVDAVTIAQAGLQNEKRPLASFLFLGPTGVGKTELAKAIAEALFDDEAAM
IRFDMSEYKQKEDVTKLIGNRATRIKGQLTEGVKQKPYCVLLLDEIEKAHSEVMDLFLQVLDDGRLTDSSGRLISFK
NTIVIMTTNIGAKKIINKWELKGNFKDLTDRDRKQFEKSMDSELQNEFRPEFLNRIENKLIFNLLERDVIEKIAEKN
LSEIADRMKRQNLTLSYEPSLIQYLSDVGTDVKNGARPLERLMKRKVLAPISVKSLQLDKSKQGYNVHLWVEGRAPD
GNHRQEQRQIHMEIEGERDNFFS

SeqID 280
MAEENAQQPSLRGKSRRERVEFARSRDILDVAHELNMELFRDGKNYRWKEHDSMVITPATNQWYWFSQRQGGDVIAL
VETIKEIGFNQAFEYLNEGTFKEFTVVNQVKEPFSYYLEPYEQLFVEARRYLKENRGLSDDTIDFFYDKGVLAQANA
KVGDMIEPVLVFKNLDKNGQVVGAALQGLVAAPDKYFGRGYLKQIMKNSQPYNGMHVDIGTPNRLVFAESSIDLMSY
YEIHKDSLSDVRLVSLEGLKTGTIGRHLIQLRAEMERRPLSSSWTDEILAQGLDEAVKQGYFKDGKNSHLLTLAVDN
DVKGKQLIEELKDKSIPVIDATPPKAEGQSKMDWNAYLQETKATFSTEKYQEKIDHLISDVILGDETYYLWHDDELV
NLGAGDSIIQAFHHQLEDRRYVINQAELYVEESSNDGATGYLSIEGHVLDKDGISDYLSDQALTDAEKVAFLETLQT
ELPDIWDEIVNHYDKVFEEVVVKYGLREKHADIIQEQELDLDPLVVPEAKEKSLEMNQETNTGGELFNRNSSFLGED
SPGTAPQPVEPTAQPDFPTNVRLHFTTDDGNMSNKAFRKNMRTLNLYANTMRDSAQWYLSEIADTTMSYVYKTPHEE
GVQVLSVHFGKKNWMHLTGVTPVYENWVDSLSEQFIDDIANSKGHFKNLKFALGTPDKLKVLNLLPEIIESDTFVFN
DLSSVQKLNNLDLSQALNPEDSDLLLLFRDEGLHQVPASLMRIKGDLEERLSHIDSGTVLGVYRERNGQLEQVSVNE
EYVKDSGQEMLSILQNKHYEEALDSGQEMVQTDGFSAEDFTKVLDAVYHVGVPDDLARVPEGVLPVWQKYLEVSEEN
QWDLEQMIDYADKNSLLVKDSAFYKEWKEDMIYKNDYHVRLQFAENWDNGVELPFRTEQLIDYKTFVTGLYEANQAH
HQRRQESQLPYTKTEFDIYAPGGQLIKDNVHYAIGDETRPVSQLMGLGYRRLPGYQELAVIDNSILSQLENKELNQE
IASEANEHSLNSQEIPKEDNYPREAFTSPKQDIKKGLAQRVEEIVAEDATKILVSSIPQVQENLSVEGNLVGTPQAD
NRMLYTNLEDFGQDYQLELAVYSPKRVDFLEDVQAPWTLALIRKEKKIGYLAYGSDWAKEFQIEEELEHLAAQIGDE
KVPEGLYKQAEVEAFIASHQGNESLQEPIPTIVAEPFDYTSASAYEISEHAFQKIREYTQSPEDLLEYMDFMSKFPQ
LSPRNVALIHEQWRGANAVATYEQWKAMGEALGIKPDDVVQTKATYVNKRTGETKEVVHQGLSVKTGEKSKITLFRP
LMVKMIPVLDENGQQLKNDKGNPKYKKLSEASLQEKALVKDGKLPVRQFQERDSKTGQPRFTTYKVFELSQTTLKPG
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ELAHATLHNPKLEKQYKELPKGQKEFEAEMTSYLLSKHFGLDTSEKAIHYMASWTDNLKALEDKQLADSLKRVHQTV
SKMLKQVEKYTNPHQLGRGKEHGLNFPKAPTKGSSR

SeqID 281
MDVSSSPNITFMLQYTEANPQYVDYTNREEAVKIDEELSLETNRQMIEGLTEDELTRIQEAVPETQLNFREYIDYMN
RSYATEEQSKELTAIFTQEADYLQKLRLIDLKNKLESAYQNGSLLWQGVISFDNAFLAEQGLYDVATGQVDQKAIKA
VMRDMMPTLIQKEGLSDSAFWWGNIHLNTDNIHIHFGLSEVESNREKIFYQPRGRMEYKGNFSQKTINRFKSGVYHG
LLKEETRSNLLRKEQILANLKADFITSIYQKDKITSSAEKNFLEQAYNHLPLNKKWRYGSNARDFAVSKFFLDRYLD
SYLNNEGSAAYQEFLKETRDFLQTYEGVYSAEKNKIYEKLRKVDGQTIRTLAESKGYDLEHHLARRVMDLRERLANN
ILRSFREAAPQIQDVQLEKNLESFSVLNQKKILEQHPEASVVKSQKAWQKLGYFVKAGEQPLEIIRPVYKSYDKHGK
GIGRPEFVSDTVYDISQLTENIQLKSLTLKDLSLFSSNELKELVDAAKLKTNPTERERRELGTYRYALKLSILESSQ
KELQVRQKLLEQVQPLASDQPFLDFKKQLIAQELQAIALQLTPNYKLSEDDKALKNRLKRQFEDSVALPVSKATPGA
IQLPIRQLWTELGLVHHIQDENILTLLKGTSTTKQAYIEELQTHISIFQLKYQINNRNKQISQLSDEATIKEMRIAN
AKGFSELKRLYDTLQPSDDGQNQISQAVSKQLQERKVIKKAQLQQTQRSGKINTDFMRQLTASLNRSQQASKKALME
RARSDEREEQEERRQAQR

SeqID 282
MKKNKFLLVSIVFIIIFVVQPQNFQSLKNIFTQNDIASQLNISSSPEEKNDGLGTAYQTQNEDLKSKSFDGQHQVIV
VNEKAQFTAEELSMRNGSWEKYDNLDFLNRVGVAEAMLGKELMPKEARQDISSVKPTGWKNKKITFNGKQDYLYNRS
HLIGFQLSGENANVKNLFTGTRALNANFNDDKSSMVYYENEVANYIKKTNHHVRYRVTPLFKNVELVARGVRIEAQS
IEDETISFDVYIFNGQPGYDIDYLTGSSEKIMITK

SeqID 283
VTKEIKIRSIPEKTWAQLHMISEEYEYPSFNEFMLAQLQRIVENGGLDLYDNKFAETLAVIKEQQAQILDQLLKNEI
KLLAYHAKQDIVEELTTDWLRFMDDVDALAAERGAGGR

SeqID 284

LLYYFIYLIKVIGNGLKLSLICGLNWLIKIVFKGQFYLFSAVFCGLLTYYMPQDIQLFTVRVLELIIMLKVIIDVTH
TALSRDFKRMKTPLFLGVMYVFFLAGNSYIKAHLLTEVMVNHLISFWLISLFFATLVIVIQPRLFKHYLLKKVIDKE
YLGIRKFTDSLPPEINLYKDADEEDADKRMRLINQNVIKHPYQEVVELSFLNREVITAIGYKAVPFEKETERTFIDD
DTIYYPIFTVHPFRNLEGKSDFYHILMKLKLSRKAAFTKNGERLLIRDF

SeqID 285
MIRNEFYNQLINSEPIGFIDPFTDLGEFDSIQMKFKQPVRNLVNKYSGKPYNLSWQNKIEQMRVLYIKYQKSLKLED
EEQEVHNRVKNKKSKKYVHEIVTTYLKLGFRFKEIEARVSLFNTRLRRNWKRSDYVTTDNPEFYLKKDLQNGYCSPN
SFLPRSMKIN

SeqID 286
MNEIKCPHCGTAFAINESEYHQLLEQIRGDAFDKEVSERLEKERLILGEQAKNQLQEVVVEKDKEIAKLQYKVKQFL
IEKDNLLKDNEYQLAEQLNQKDMMLRDLENQIDRLRLEHENSLQEALTKVERERDAIQNQLHIQEKEKDLALASVKS
DYEVQLKAANEQVEFYKNFKAQQSTKAVGESLEHYAETEFNKVRHLAFPNAYFEKDNTLSSRGSKGDFIYREKDEND
LEFLSIMFEMKNESDDTIKKHKNEDFFKELDKDRREKSCEYAVLVTMLEADNDYYNTGIVDVSHKYPKMYVIRPQFF
IQLIGILRNAALNTLKYKQELALMKEQNIDITHFEEDLDIFKNAFAKNYNSASKNFQKAIDEIDKSIKRMEAVKAAL
TTSENQLRLANNKLDDVSVKKLTRKNPTMKAKFDALKD

SeqID 287
MNHFELFKLKKAGLTNLNINNIINYLKKNSLTSLSVRNMAVVSKCKNPTFFIENYKQLDLKKLRQEFKKFPVLSILD
SNYPLELKEIYNPPVLLFYQGNIELLSKPKLAVVGARQASQIGCQSVKKIIKETNNQFVIVSGLARGIDTAAHVSAL
KNGGSSIAVIGSGLDVYYPTENKKLQEYMSYNHLVLSEYFTGEQPLKFHFPERNRIIAGLCQGIVVAEAKMRSGSLI
TCERALEEGREVFAIPGNIIDGKSDGCHHLIQEGAKCIISGKDILSEYQ

SeqID 288
MTERTFEDIELDLKLFQIKLDNAENSKRLLQKLKNDVMELQIELLESLKLGDAYLTESEELEENNDFILTVNSETLS
LSYDNRINLVSKEIMDYENALDKLYYEKQSLMQKSNERKGG

SeqID 289
LFNKIGFRTWKSGKLWLYMGVLGSTIILGSSPVSAMDSVGNQSQGNVLERRQRDAENRSQGNVLERRQRDAENRSQG
NVLERRQRDAENRSQGNVLERRQRDAENRSQGNVLERRQRDVENKSQGNVLERRQRDAENKSQGNVLERRQRDAENR
SQGNVLERRQRDVENKSQGNVLERRQRDAENRSQGNVLERRQRDVENKSQGNVLERRQRDVENKSQGNVLERRQRDA
ENRSQGNVLERRQRDVENKSQGNVLERRQRDVENKSQGNVLERRQRDAENRSQGNVLERRQRDAENRSQGNVLERRQ
RDAENKSQVGQLIGKNPLLSKSIISRENNHSSQGDSNKQSFSKKVSQVTNVANRPMLTNNSRTISVINKLPKTGDDQ
NVIFKLVGFGLILLLTSRCGLRRNEN

SeqID 290
MKRLTYYFKGYIKETIFGPLFKLLEASFELLVPIVIAKMIDETIPRGDRSSLLLQIGLIFFLAAVGVVVAITAQYYS
SKAAVGYTRQLTEDLYQKVMSLGKKDRDELGTASLITRLTADTFQIQTGLNQFLRLFLRAPIIVFGAIIMAFSISPS
LTIWFLVMVVTLFIIVFVMSRLLNPIYLKIRTSTDYLVKLTRQQLQGVRVIRAFNQVDRESEAFNDINYHYTNLQLK
AGRLSSLVTPLTFLVVNITLVVIIWRGNLNIANHLLSQGMLVALINYLLQILVELLKMTMLVTSLNQSYISAKRIIA
VFERPSEIIDDKLEPKYSNKALEVQEMAFSYPNSSEKALSDITFSMNVGETLGIIGGTGSGKSTLVNLLLHIYKVQE
GDIDIYHQGKSPDTISNWRTLVRVVPQNAQLFKGTIRSNLSLGLGKVSEEKLWTALEIAQASDFVKEKDGQLDAPVE
SFGRNFSGGQRQRLTIARALVQDKIPFLILDDATSALDYLTEARLFKAITKHFNQTNLIIVSQRINSIQNADRILLL
DKGKQVGFDNHQSLLAHNKVYKSIYHSQNFKEEE

SeqID 291
MKFNEQSNSQAALLGLQHLLAMYAGSILVPIMIASALGYNAEQLTYLIATDIFMCGIATLLQLQLSKHFGVGLPVVL
GCAFQSVAPLSIIGAQQGSGYMFGALIASGIYVVLVAGIFSKVANFFPPIVTGSVITTIGLTLIPVAMGNMGDNAKE
PSLQSLTLSLVTIGVVLLINIFAKGFLKSISILIGLISGTILAAFMGLVDASVVAEAPLVHIPKPFYFGAPRFEFTS
ILMMCIIATVSMVESTGVYLALSDITNDKLDSKRLRNGYRSEGLAVLLGGLFNTFPYTGFSQNVGLVQISGIRTRKP
IYFTALFLVILGLLPKFGAMAQMIPSPVLGGAMLVLFGMVALQGMKMLNQVDFEHNEHNFIIAAVSIAAGVGFNGTN
LFISLPNTLQMFLTNGIVISTLTAVVLNIILNGLPKKLI

SeqID 292
MANTYDLISQRIEAQRQKLIAIDIVAVASSLGLNLKQGSGGHLYWDEHDSFHIYPQTNTFRWWSRSMGTNTIDLVQV
IQEELTGKKPSFRETVNFLETGQFESVTVTPVVREPFKHYLAPYEHHNFDLGRQYLKEERGLSDETIDFALASGSMS
SATLKKGDYFEPVIIFKSFAEDGRMIGGSLQGIVENKVQHPERGRLKQIMKHSDGLAGFHLDVGTPKRLVFSEAPID
LLSYYELHKESLQNVRLVAMDGVKKGVISRYTADLLTDGQYSQTMPRESIRGAIDAINQTTRILKNNPNMITIAVDN
DEAGRNFIKELQEDGIPINVDLPPRKEHQSKMDWNNYLKQKKGLLKMPQTEGTQKAPEQVLEHEKMDRSQISSGSLE
DDPQGSAKPVSKRDTFEQAVTSHPTFSYPLLQFSTEEAFVSNVRDGYHIASEEDIRNLNYYAPSLQQTANWYRDNLA
DRQVTYMLKGDKEIKALQVSFAKDKFAHLTGIRPIGKGLSAEKLLDDFAKGRGSYPNLTLSNGFNDKIQVLPMIQEL
SQSKSFVFTDLEEVQKMRNLKASHAIQSNNRSLVVALKTIDDVTFPSSLLRGKKNLNDDLIQKAKENEVLGVLSEKD
GNITVLSVNDKYIQDGGQALKDMIKNGELEPLQMETIQRHVPHENAYPKDSDGDGLTDDEEIALGTNPFSSDSDGDG
TPDNVEKANGTDPTNASDNEVTRQQEANKRDFTLSEMIKAKNTAALNQHLQDGIKQYFDSDTYKQYLEGMAHFNNYS

PRNIQLIMSQFPEASMVASFQEWRKRNGSVKKGEKAIYIQAPVSVMKKDENGKPILNPETGEKETITYFKPVPVFDI
KQVSPQEGKELNLPKAMGTIPEQLDKEYYQNVYRSLRDISQNNNKVPIRFRELGQEDGFYSPQTNEIVIKKGMSYER
TLSTLIHEMAHSELHNKQSLTERFDGKLTRSTKELQAESIAYVVSSHLGFDTSQESFPYLASWSKEKDGLANLTAQL
EIVQEEAKNLMERIDQQLSQYQTVTLNKETQQLTKQEMKKQTHPFYQSLAAAKTSRAQVTTQEKEASVKKDNRPTMP

SeqID 293
MNSQETKGHGFFRKSKAYGLVCGIALAGAFTLATSQVSADQVTTQATTQTVTQNQAETVTSTQLDKAVDTAKKAAVA
VTTTTAVNHATTTDAQADLANQTQAVKDVTAKAQANTQAIKDATAENAKIDAENKAEAERVAKANKAGQAEVDARNK
AGQAAVDARNKAKQQAQDDQKAKIDAENKAESQRVSQLNAQNKAKIDAENKDAQAKADATNAQLQKDYQTKLANIKS
VEAYNAGVRQRNKDAQAKADATNAQLQKDYQAKLALYNQALKAKAEADKQSINNVAFDIKAQAKGVDNAEYGNSIMT
AKTKPDGSFEFNHDMIDGVKTIGYGKLTGKVNHHYVANKDGSVTAFVDSVTLYKYEYRNVAQNAAVNQNIVFRVLTK
DGRPIFEKAHNGNKTFAETLNKTLQLNLKYELKPHASSGNVEVFKIHDDWVHDTHGSALVSYVNNNDAVPNVVIPEQ
PTPPKPEKVTPEAEKPVPEKPVEPKLVTPVLKTYTPVKFIPREYKPVPSTPETFTPEKFTPAQPKVKPHVSVPEKIN
YKVAVHPVQIPKATPTKKVLDENGQSINGKSVLPNATLDYVAKQNFSQYKGIKASAEAIAKGFAFVDQPNEALAELT
VKSIKASNGDDVSSLLEMRHVLSKDTLDQKLQSLIKEAGISPVGEFYMWTAKDPQAFYKAYVQKGLDITYNLSFKVK
KEFTKGQIKNGVAQIDFGNGYTGNIVVNDLTTPEVHKDVLDKEDGKSINNGTVKLGDEVTYKLEGWVVPANRGYDLF
EYKFVDHLQHTHDLYLKDKVVAKVAITLKDGTVIPKGTNLVQYTETVYNKETGRYELAFKADFLAQVSRSSAFGADD
FIVVKRIKAGDVYNTADFFVNGNKVKTETVVTHTPEKPKPVMPQKVTPKAPALPSTGEQGVSVLTVLGAALLSLLGL
VGFKKRQQ

SeqID 294
MNQIKIITGLTVATLSAVVGNVYAEDITPTAPVNEPQVSSETAKTPQVTESQVNSAKVTADQATSDVNAQKNVVNNA
QNQKNQAQQKLVNATTTLNETQKLVQESTNQNQVQQTVDSAKQRLSQTEANQKITQTEQVKAQNQVNAQQTVVVNNE
HDVATKTADVKQAQASVDTAKDALTNTIVNSDLNKAQSNVTTKTADVKTATDALTKAQATDKTLTNQKAKAQQIVDS
AKQNLSAKDTQLSQANAEVNHHKFKTALGQSHYYNQRDNAWAGVYGGHTFASTGCVPSALAMVYSDLSNRTITPREI
ADYLYNNTDEFNKRFGGTSGKGIISATKAFGYVVTHLASKNAITEALKAGHHVVAAVQNNKFSPWGPQYSHEIVLRG
SSNGNTYVYDPYNRDNNGFYSVDRIWNEQSRDSIDTAGVGVPFFAIMTKNMANALTKQSQALASQQVAQKQLNDAQA
KATGLNAVTMQTPIAQANLIKAQSNLKDAQKRLAEAQASVKLANQDNVKKQADLTKAESKLKDAQKQLAAAQAKLTT
SKTKLNQLKQVLAEASQQVAQANQDYKQAKDNLTQKTAYLTNLRNAQANLIKAQSDVAQAKDNLANKIAKLQREVAY
LQELKTKAVDAQSQYQKVLSAYKSVLSAKASLKLAEEKARLDKKGHEAVAVVDETGKITSYITSKHKIEMKSLVATK
TTDVKQVSVAKASVLPSTGDVKQVSVALLGMLLTFSGFLGIRKQSKKVIN

SeqID 295
MISRKVALVTGASAGFGAAIVTKLVSDGYSVIGCARRMDKLKCFGEKFSEGYFYPLQMDITSRESVDKALESLPKNL
QSIDLLVNNAGLALGLDKSYEADFEDWMTMINTNVVGLIYLTRCILPKMVEVNRGLIINLGSTAGTIPYPGANVYGA
SKAFVKQFSLNLRADLAGTKIRVTNLEPGLCEGTEFSTVRFKGDHKRVEKLYEGAHAIQAEDIANTVSWVASQPEHI
NINRIEIMPVSQTYGPQPVYRD

SeqID 296
MIYLDNAATTALTPSVIEKMTNVMTSNYGNPSSIHTFGRQANQLLRECRQIIAEYLNVNSREIIFTSGGTESNNTAI
KGYALANQLKGKHIITSEIEHHSVLHTMTYLSERFGFDITYLKPNHGQITAKDVQEALRDDTIMVSLMFANNETGDF
LPIQEIGQLLRNHQAVFHVDAVQVFSKMELDPHSLGIDFLAASAHKFHGPKGVGILYCAPHHFDSLLHGGDQEEKRR
ASTENIIGIAGMSQALTDATTNTLKNWTHISQLRTTFLDAISDLDFYLNNGQDCLPHVLNIGFPRQNNGLLLTQLDL
AGFAVSTGSACTAGTVEPSHVLTSLYGANSPRLNESIRISFSELNTQEEILELAKTLRKIIGD

SeqID 297
MSRKTFKHILSIGVCTLVLSMSLYYTEKAHAIAGPSDRQYVENPNPHIIVNVTGTDQNGNSILPHYIEVNVKMGQTL
SKEEILDYIARNLNSSVGGESKNVQYSNIEFKESAYLKRQLDDGKTEEIAIDNDGVTVPKDGPNKFWIDVPVTCTVT
PIVTETHEVRWGTPVAISHRIYFVEESSGKVLDEYTNLHTADSELNGYRVGDYITDYALSKSAYEAFLNSRLDKEGY
KLQHRISTNVRQNLQIDKLIFNYDFNEENIYYQIGNIRPLLSRSSAEVESDIITERYYVSKNAKSLARTESTISIKM
VDAKIEQPLFNHTLTGYQLATVSHVYNRLFEENLIPTTKSGERYFIQNMKKTAEQEYTVYLSETPYSKENAPVISYD
ARPVDWDYHSGASGSLENQPNIYTEEDSTEFLGNKPQAACYPNKQFACENTDSKYNYSYLEK

SeqID 298
MNPLIIGMNDKQAEAVQTTDGPLLIMAGAGSGKTRVLTHRIAYLIDEKYVNPWNILAITFTNKAAREMRERAIALNP
ATQDTLIATFHSMCVRILRREADYIGYNRNFTIVDPGEQRTLMKRIIKQLNLDTKKWNERSILGTISNAKNDLLDEI
AYEKQAGDMYTQVIAKCYKAYQEELRRSEAMDFDDLIMMTLRLFDQNKDVLAYYQQRYQYIHVDEYQDTNHAQYQLV
KLLASRFKNICVVGDADQSIYGWRGADMQNILDFEKDYPQAKVVLLEENYRSTKKILQAANNVINHNKNRRPKKLWT
QNDEGEQIVYHRANNEQEEAVFVASTIDNIVREQGKNFKDFAVLYRTNAQSRTIEEALLKSNIPYTMVGGTKFYSRK
EIRDVIAYLNILANTSDNISFERIVNEPKRGVGPGTLEKIRSFAYEQNMSLLDASSNVMMSPLKGKAAQAVWDLANL
ILTLRSKLDSLTVTEITENLLDKTGYLEALQVQNTLESQARIENIEEFLSVTKNFDDNPEITVEGETGLDRLSRFLN
DLALIADTDDSATETAEVTLMTLHAAKGLEFPVVFLIGMEEGVFPLSRAIEDADELEEERRLAYVGITRAEQILFLT
NANTRTLFGKTSYNRPTRFIREIDDELIQHQGLARPVNSSFGVKYSKEQPTQFGQGMSLQQALQARKSNSQPQVTAQ
LQALNANNSHETSWEIGDVATHKKWGDGTVLEVSGSGKTQELKINFPGIGLKKLLASVAPISKKEN

SeqID 299
MKLYVQLMVILTFSFAGEVLSTIFNLPVPGSIIGLILLFLALKYKIIRLRHIDAVGNFLLANMTILFLPPAVGLMEH
FQDIKPYLFGIAIIILGALFLNILTIGLVSQWIKKRYEGDYPEIGGKNGNFNE

SeqID 300
MIFVTVGTHEQQFNRLIKEVDRLKGTGAIDQEVFIQTGYSDFEPQNCQWSKFLSYDDMNSYMKEAEIVITHGGPATF
MSVISLGKLPVVVPRRKQFGEHINDHQIQFFKKIAHLYPLAWIEDVDGLAEVLKRNIATEKYQGNNDMFCHKLEKII
GEI

SeqID 301
MVMKIIELKEATVQVSNGLAEMKTILDHVNLSIYEHDFITILGGNGAGKSTLFNVIAGTLMLSGGNIYIMGQDVTNL
PAEKRAKYLSRVFQDPKMGTAPRMTVAENLLVAKFRGEKRPLVPRKITNYTEEFQKLIARTGNGLDRHLETPTGLLS
GGQRQALSLLMATLKKPNLLLLDEHTAALDPRTSVSLMGLTDEFIKQDSLTALMITHHMEDALKYGNRILVMKDGKI
VRDLNQAQKNKMAIADYYQLFD

SeqID 302
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STATATATHSTMKQVVDNQTQNKELVKNGDFNQTNPVSGSWSHTSAREWSAWIDKENTADKSPIIQRTEQGQVSLSS
DKGFRGAVTQKVNIDPTKKYEVKFDIETSNKAGQAFLRIMEKKDNNTRLWLSEMTSGTTNKHTLTKIYNPKLNVSEV
TLELYYEKGTGSATFDNISMKAKGPKDSEHPQPVTTQIEESVNTALNKNYVFNKADYQYTLTNPSLGKIVGGILYPN
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HYSNHFWPTVNPYKMAGTTEKDAKREDTTKEFMSKHSKDAKEKTGQVTGTSDFVGSVKLNDHFALAAMDFTNWDRTL
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SeqID 303
MHSFSNPGYPYDNAVTEAFFKYLKHRQINRKHYQNIKQVQLDCFEYIENFYNNYNPHTANLGLTPNQKEENYFNAIK

SeqID 304
VKKTYGYIGSVAAILLATHIGSYQLGKHHMGLATKDNQIAYIDDSKGKAKAPKTNKTMDQISAEEGISAEQIVVKIT
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IAEQVAKGTKEAKEKGLAQVAHLSKEEVAAVNEAKRQGRYTTDDGYIFSPTDIIDDLGDAYLVPHGNHYHYIPKKDL
SPSELAAAQAYWSQKQGRGARPSDYRPTPAPGRRKAPIPDVTPNPGQGHQPDNGGYHPAPPRPNDASQNKHQRDEFK
GKTFKELLDQLHRLDLKYRHVEEDGLIFEPTQVIKSNAFGYVVPHGDHYHIIPRSQLSPLEMELADRYLAGQTEDND
SGSEHSKPSDKEVTHTFLGHRIKAYGKGLDGKPYDTSDAYVFSKESIHSVDKSGVTAKHGDHFHYIGFGELEQYELD
EVANWVKAKGQADELAAALDQEQGKEKPLFDTKKVSRKVTKDGKVGYMMPKDGKDYFYARDQLDLTQIAFAEQELML
KDKKHYRYDIVDTGIEPRLAVDVSSLPMHAGNATYDTGSSFVIPHIDHIHVVPYSWLTRDQIATVKYVMQHPEVRPD
VWSKPGHEESGSVIPNVTPLDKRAGMPNWQIIHSAEEVQKALAEGRFATPDGYIFDPRDVLAKETFVWKDGSFSIPR
ADGSSLRTINKSDLSQAEWQQAQELLAKKNTGDATDTDKPKEKQQADKSNENQQPSEASKEEKESDDFIDSLPDYGL
DRATLEDHINQLAQKANIDPKYLIFQPEGVQFYNKNGELVTYDIKTLQQINP

SeqID 305
MKKGFFLMAMVVSLVMIAGCDKSANPKQPTQGMSVVTSFYPMYAMTKEVSGDLNDVRMIQSGAGIHSFEPSVNDVAA
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VNIAKELGRLDPKHKDSYTKKAKAFKKEAEQLTEEYTQKFKKVRSKTFVTQHTAFSYLAKRFGLKQLGISGISPEQE
PSPRQLKEIQDFVKEYNVKTIFAEDNVNPKIAHAIAKSTGAKVKTLSPLEAAPSGNKTYLENLRANLEVLYQQLK

SeqID 306
LRKKQKLPFDKLAIALMSTSILLNAQSDIKANTVTEDTPATEQTVETPQPTAVSEEAPSSKETKTPQTPSDAGETVA
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DLEKAKKEHGITYGEWVNDKVAYYHDYSKDGKTAVDQEHGTHVSGILSGNAPSETKEPYRLEGAMPEAQLLLMRVEI
VNGLADYARNYAQAIRDAINLGAKVINMSFGNAALAYANLPDETKKAFDYAKSKGVSIVTSAGNDSSFGGKTRLPLA
DHPDYGVVGTPAAADSTLTVASYSPDKQLTETATVKTADQQDKEMPVLSTNRFEPNKAYDYAYANRGTKEDDFKDVK
GKIALIERGDIDFKDKIAKAKKAGAVGVLIYDNQDKGFPIELPNVDQMPAAFISRKDGLLLKDNPQKTITFNATPKV
LPTASGTKLSRFSSWGLTADGNIKPDIAAPGQDILSSVANNKYAKLSGTSMSAPLVAGIMGLLQKQYETQYPDMTPS
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DKPQELYYQATVQTDKVDGKHFALAPKALYETSWQKITIPANSSKQVTVPIDASRFSKDLLAQMKNGYFLEGFVRFK
QDPKKEELMSIPYIGFRGDFGNLSALEKPIYDSKDGSSYYHEANSDAKDQLDGDGLQFYALKNNFTALTTESNPWTI
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EVLDKEGNVVWTSEVTEQVVKNYNNDLASTLGSTRFEKTRWDGKDKDGKVVANGTYTYRVRYTPISSGAKEQHTDFD
VIVDNTTPEVATSATFSTEDRRLTLASKPKTSQPVYRERIAYTYMDEDLPTTEYISPNEDGTFTLPEEAETMEGATV
PLKMSDFTYVVEDMAGNITYTPVTKLLEGHSNKPEQDGSDQAPDKKPEAKPEQDGSGQTPDKKTETKPEKDSSGQTP
GKTPQKGQPSRTLEKRSSKRALATKASTRDQLPTTNDKDTNRLHLLKLVMTTFFLGLVAHIFKTKRQKETKK

SeqID 307
FSVTYSQSERTVVFSFGEITFSRSRWTNGFETRIPVDEWLGLEKYKRYSIEFLYHVAKLATMMPYRQVCKVIDSTLQ
TIITKDCVLKAVKFVEKLLKEKERYRFYLEEPPERKKVKKLYVEGDGVMIKSTDSREERRYLDLTHFVIHTGSKKVS
TKRYELQDKHEILQLNYDKAKYNLLDYIYNNYEVDDDTILITNSDMGKGYTSRVFKELGKALKVKKHEHFWDIYHVK
EKLSSYLRKYPIELTDFALDAVKKYNSDKLELVFDTVESLICDELEDQEFQKFKKKVLNNFKYIKPAHLRNLSNRGI
GIMESQHRKITYRMKRRGMYWSKWGISTMANMIILERANGLRELFFGSWRKVYSEYKEGSFSAGRLFKKTDELDKFS
KPLLKNGRKWSITGIKTK

SeqID 308
KASSGDYGTKREIITANKDKYSISKMCRWLNMPRSSYYYQAVESVSKTEFEETIKRIFLDSESRYGSRKIKICLNNE
GITLSRRRIRRIMKRLNLVSVYQKATFKPHSRGKNEAPIPNHLDRQFKQERPLQALVTDLTYVRVGNRWAYVCLIID
LYNREIIGLSLGWHKTAELVKQAIQSIPYALTKVKMFHSDRGKEFDNQLIDEILEAFGITRSLSQAGCPYDNAVAES
TYRAFKIEFVYQETFQSLEELALKTEKATLFCTTFIKCCLFRFLPML

SeqID 309
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MVQFENLQACEVGFNVYFKADNLKEVK

SeqID 310
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SPLVREVLHHYPLGSRVTYKGQEFEILSIEAADMDNLIRIELQNDFSYLIEQNPVLYFQNLAEIRQVLHLSRSEIVE
REEQPEEEWTLFSFMDEGTEDNEKEPEIVFESTDKVVTLDSQPAQVSETISESVPETKEVIEAEQAIAVDFSFPEDL
TNFYPKTARDKVVANLVAIRLVKELESANQSATPNEQEILAKYVGWGGLANEFFDEYNPKFSKEREELKTLVSDKEY
SDMKQSSLTAYYTDPLLIRQMWSKLEQDGFSGGKILDPSMGTGNFFAAMPAHLREKSELCGVELDTITGAIAKQLHS
NVHIEVKGFETVAFNDNSFDLVISNVPFANIRIADNQYDKPYMIHDYFVKKSLDLVHDGGQVAIISSTGTMDKRTEN
ILQDIRETTDFLGGVRLPDTAFKAIAGTSVTTDMLFFQKHLNKGYQADDIAFSGSVRYDKDERIWLNPYFDGEYNAQ
VLGRYEIKNFNGGTLSVKETTDNLIASVREALQHVKAPRVIDKTEVMINSDVIARQVIDTTIPPEIRENLEQYSYGY
KGSTIYYRDNKGIRVGTKTEEISYYVDDEGNFQAWESKHSQKQIDRFNNLEVTDSTALDVYVTEEPAKRGQFKGYFK
KAVFYEAPLSEKEVARIKGMVDIRNAYQEVIAIQRNYDYDKNEFNRLLGNLNRTYDSFVKRYGFLNSPVNRNLFDSD
DKYSLLASLEDESLDPSGKTVIYTKSLAFEKALVRPEKMVKEVSTALDALNSSLADGRGVDFDYMASIYQTASKAAL
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GSRWIPLAVYGKFVQEAFMGKNYDLTATEVEEVLSVSPIDGTMEFRTRFAYTYSTATDRSLGVAGSRYDSGRKIFEN
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DMRLIDPAYTLSDNQKIMQVVDNVERIYREGKGDKATQMIFSDIGTPKSKEEGFDVYNELKALLVDRGIPKEEIAFV
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YITKGSFDNYLWATQENKLRYIKQIMTSKDPVRSAEDIDEQTMTASDFKALATGNPYLKLKMELENELTVLENQKRA
FNRSKDEYRHTISYCEKNLPVMEKRLRQYDRDIEKSQATKNQEFIMRFDNQTIDNRSEAGDYLRKLITYNRSETKEV
RTLATFRGFELKMATRSPGEPLSDMVSLTISGDNQYSVSLDLKSDVGTIQRINNAIDHILEDKEKTEEMTNNLKDKL
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SeqID 311
MTTKCNHHFLINQEKGEKYVFRKSKQYRTLCSVALGTVVMAFVALAGPMVQADEVGRTVATSVQTETNPATNLKENQ
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TISKTVEIYKSDKATYEAEKKWVEKRNEELTAAYDKAEQTGTGLNHSVDTTVSELKSQDQNAHVTVNTQTVKSGDGT
SVSGYQEYVKSVAAIDKKNKANLADYRTKKQAADAVVAKNQLIQKENEAGLAKAKAENEAIDRRNKEGQKAVDEANK
AGQAAVEQANQEKQKQAANRAFEIATITKRNKEREEVAKKENAAIDAYNAKEWIRYKRDLANISKGEEGYISEALAQ
ALDLNHGEPQVKHGAGTRNPDRIISKGDAMLGGYSNILDSTGFFVYNHFKTGETLNFTYQNLKHARFDGKKITAITY
DITNLVSPTGTNAVQLVVPNDPTEGFIAYRNDGAGNWRTDKMEFRVKARYFLEDGSQVTFTKEKPGVFTHSSLNHND
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SLIFEDYLPAGYAFDMTKTQAENSDYDLTFDKNKNFVTLKAKDSLLQTLNKELNKSYQLSAPKLYGSVQNDGATYSN
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KDTIARGFLFVDDYPEEALDLVDKGTVITTLDGKAVSGISVYSYTSLDKAPKELQDKLARANISPKGAFQVFEPDNH
QAFYDTYVKTGQSLALLTKMKVKDSLYGQTVRYKNKAYQVDFGNGYETKEVVNTVVHPEPKKQNLNKDKVDINGKAM
LVGSQNFYTLSWDLDQYRGLQADKSQIAQGFYFVDDYPEDVLLPDTKAIQIMTKDGKAVKGMEIKTYHQLSDAPKEL
QAALAKRNITPKGAFQVFMPKDPQAFYKAYVTTGQNLTIVNPMTVREAVYNSGKSYDNVAYQVDFGQAYETNIVTNH
VPTVNPHKSNTNKEGVSIEGKTVLPNTVNYYKIVLDYSQYKNMIVTDDVLVKGFYMVDDYPEEALTPHPDGTQVMDQ
NGKFVRGLSVRTYASLADAPKAVQEAMKSRNFIPKGAIQVFQADDPKTFFETYVKTGQKLVVTTPMTVKNERIQTGG
QYENTAYQIDFGIAYVTETVVNNVPKLEPQKDVVIDLSQKDKSLNGKAIALDQVFNYRLVGSLIPRNRATALVEYSF
KDDYDEKHDEYKGVYKAYTLRDVTLKDGTVLKQGTEVTKYTLQSVDKAKGTIAIRFDTAFLENIADESEFQAELYLQ
MKRIASGDVENTVIHSVNGYNIRSNTVKTTTPQPESPTPDNPPSPQPPVPTTESPVQASVLPSTGESQSLLALIGGG
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SeqID 312
MANNNFYNRDPFGNMDDIFNSLMGNMGGYNSENKRYLINGREVTPEEFSQYRQTGKLPGQELNNQNTPTNQVSADSV
LTKLGTNLTDQARQHLLDPVIGRNKEIQETAEILARRTKNNPVLVGDAGVGKTAVIEGLAQAIINGDVPAAIKNKEI
ISIDISSLEAGTQYRGSFEENIQNIIKEVKETGNIILFFDEIHQILGAGSTGGDSGSKGLADILKPALSRGELTVIG
ATTQDEYRNTILKNAALARRFNEVKVNAPSAQDTFNILMGIRNLYEQHHNVVLPDSVLKAAVDLSIQYIPQRSLPDK
AIDLIDMTAAHLAAQHPVTDLKSLEKEIAAQRDKQEKAVNTEDFEEALKVKTRIEELQNQIDNHTEGQKVTATINDI
AMSIERLTGVPVSNMGASDIERLKELGNRLKGKVIGQNDAVEAVARAIRRNRAGFDDGNRPIGSFLFVGPTGVGKTE
LAKQLAFDMFGSKDAIVRLDMSEYNDRTAVSKLIGATAGYVGYDDNSNTLTERIRRNPYSIVLLDEIEKADPQVITL
LLQVLDDGRLTDGQGNTINFKNTVIIATSNAGFGNEAFTGDSDKDLKIMERISPYFRPEFLNRFNGVIEFSHLSKDD
LNEIVDLMLDEVNQTIGKKGIDLVVDENVKSHLIDLGYDEAMGVRPLRRVIEQEIRDRITDYYLDHTDVKHLKANLQ
DGQIVISER

SeqID 313
MGRFKELLESKKALILHGALGTELESRGCDVSGKLWSDKYLIEDPAAIQTIHEDYIRAGADIVTTSTYQATLQGLAQ
VGVSESQAEDLIRLTVQLAKAVREQVWKSLTKEEKSERIYPLISGDVGPYAAFLADGSEYTGLYDIYKEGLKNFHRH
RIELLLLDEGVDLLALETIPNAQEAEALIELLVEDFPQVEAYMSFTSQDGKTISDGSAVAGLAKAIDVSPQVVALGIN
CSSPSLVADFLQAIAEQTDKPLVTYPNSGEIYDGASQSWQSSRDHSHTLLENTSDWQKLGAQVVGGCCRTRPADIAD
LSEHLT

SeqID 314
MKIGIDKIGFATSQYVLEMTDLAIARQVDPEKFSKGLLLDSLSITPVTEDIVTLAASAANDILSDEDKETIDMVIVA
TESSIDQSKAASVYVHQLLEIQPFARSFEMKEACYSATAALDYAKLHVEKHPDSKVLVIASDIAKYGIKSTGESTQG
AGSIAMLISQNPSILELKEDRLAQTRDIMDFWRPNYSDVPYVNGMFSTKQYLDMLKTTWKEYQKRFNTSLSDYAAFC
FHIPFPKLALKGFNKILDNNLDEQKKAELQENFEHSITYSKKIGNCYTGSLYLGLLSLLENSQNLKAGDQIAFFSYG
SGAVAEIFTGQLVDGYQNKLQSDRMDQLNKRQKITVTEYEKLFFEKTILDENGNANFNTYRTGTFSLDSICEHQRIY
KKINN

SeqID 315
MKSAYIFFNPKSGKDEQALAQEVKSYLIEHDFQDDYVRIITPSSVEEAVALAKKASEDHIDLVIPLGGDGTINKICG
GVYAGGAYPTIGLVPAGTVNNFSKALNIPQERNLALENLLNGHVKSVDICKVNDDYMISSLTLGLLADIAANVTSEM
KRKLGPFAFVGDAYRILKRNRSYSITLAYDNNVRSLRTRLLLITMTNSIAGMPAFSPEATIDDGLFRVYTMEHIHFF
KLLLHLRQFRKGDFSQAKEIKHFHTNNLTISTFKRKKSAIPKVRIDGDPGDQLPVKVEVIPKALKFIIPNSLP

SeqID 316
MTTFTAKFIDQEWEVPVESGRYHMIVGEFCPYAQRPQIARQLLGLDKHISISFVDDVPSDIGLIFSQPEQVTGAKSL
RDIYHLTDPTYKGPYTIPILIDKTDNRIVCKESADMLRLFTTDFSDLHQEDAPVLFSQETASLIDNDIKDINNNFQS
LMYKLAFLDKQADYDTYSKEFFTFLDQKEHLLGQRPFLLGDNLSEVDIHFFTPLVRWDIAGRDLLLLNQKALEDYPN
IFSWAKTLYNDFNLKTLTNPQSIKNNYYLGKFGRAVRHHTIVPTGPNMVKWEK

SeqID 317
MKKKIILKSSVLGLVAGTSIMFSSAFADQVGVQVIGVNDFHGALDNTGTANMPDGKVTNAGTAAQLDAYIDDAQKDF
KQTNPNGESIRVQAGDMVGASPANSGLLQDEPTVKTFNAMNVEYGTLGNHEFDEGLAEYNRIVTGKAPAPDSNINNI
TKSYPHEAAKQEIVVANVIDKVNKQIPYNWKPYAIKNIPVNNKSVNVGFIGIVTKDIPNLVLRKNYEQYEFLDEAET
IVKYAKELQAKNVKAIVVLAHVPATSKDDIAEGEAAEMMKKVNQLFPENSVDIVFAGHNHQYTNGLVGKTRIVQALS
QGKAYADVRGVLDTDTQDFIETPSAKVVAVAPGKKTGSADIQAIVDQANTIVKQVTEAKIGTAEVSGMITRSVDQDN
VSPVGSLITEAQLAIARKSWPDIDFAMTNNGGIRADLLIKPDGTITWGAAQAVQPFGNILQVVEITGRDLYKALNEQ
YDQKQNFFLQIAGLRYTYTDNKEGGEETPFKVVKAYKSNGEEINPDAKYKLVINDFLFGGGDGFASFRNAKLLGAIN
PDTEVFMAYITDLEKAGKKVSVPNNKPKIYVTMKMVNETITQNDGTHSIIKKLYLDRQGNIVAQEIVSDTLNQTKSK
STKINPVTTIHKKQLHQFTAINPMRNYGKPSNSTTVKSKQLPKTNSEYGQSFLMSVFGVGLIGIALNTKKKHMK

SeqID 318
MAYIWSYLKRYPNWLWLDLLGAMLFVTVILGMPTALAGMIDNGVTKGDRTGVYLWTFIMFIFVVLGIIGRITMAYAS
SRLTTTMIRDMRNDMYAKLQEYSHHEYEQIGVSSLVTRMTSDTFVLMQFAEMSLRLGLVTPMVMIFSVVMILITSPS
LAWLVAVAMPLLVGVVLYVAIKTKPLSERQQTMLDKINQYVRENLTGLRVVRAFARENFQSQKFQVANQRYTDTSTG

LFKLTGLTEPLFVQIIIAMIVAIVWFALDPLQRGAIKIGDLVAFIEYSFHALFSFLLFANLFTMYPRMVVSSHRIRE
VMDMPISINPNAEGVTDTKLKGHLEFDNVTFAYPGETESPVLHDISFKAKPGETIAFIGSTGSGKSSLVNLIPRFYD
VTLGKILVDGVDVRDYNLKSLRQKIGFIPQKALLFTGTIGENLKYGKADATIDDLRQAVDISQAKEFIESHQEAFET
HLAEGGSNLSGGQKQRLSIARAVVKDPDLYIFDDSFSALDYKTDATLRARLKEVTGDSTVLIVAQRVGTIMDADQII
VLDEGEIVGRGTHAQLIENNAIYREIAESQLKNQNLSEGE

SeqID 319
MKKGQVNDTKQSYSLRKYKFGLASVILGPFIMVTSPVFADQTTSVQVNNQTGTSVDANNSSNETSASSVITSNNDSV
QASDKVVNSQNTATKDITTPLVETKPMVEKTLPEQGNYVYSKETEVKNTPSKSAPVAFYAKKGDKVFYDQVFNKDNV
KWISYKSFCGVRRYAAIESLDPSGGSETKAPTPVTNSGSNNQEKIATQGNYTFSHKVEVKNEAKVASPTQFTLDKGD
RIFYDQILTIEGNQWLSYKSFNGVRRFVLLGKASSVEKTEDKEKVSPQPQARITKTGRLTISNETTTGFDILITNIK
DDNGIAAVKVPVWTEQGGQDDIKWYTAVTTGDGNYKVAVSFADHKNEKGLYNIHLYYQEASGTLVGVTGTKVTVAGT
NSSQEPIENGLAKTGVYNIIGSTEVKNEAKISSQTQFTLEKGDKINYDQVLTADGYQWISYKSYSGVRRYIPVKKLT
TSSEKAKDEATKPTSYPNLPKTGTYTFTKTVDVKSQPKVSSPVEFNFQKGEKIHYDQVLVVDGHQWISYKSYSGIRR
YIEI

SeqID 320
MENWKFALSSILGHKMRAFLTMLGIIIGVASVVLIMALGKGMKDSVTNEITKSQKNLQIYYKTKEDQKNEDNFGAQG
AFMQGSDTNRKEPIIQESWLKKIAKEVDGVSGYYVTNQTNAPVAYLEKKAKTVNITGVNRTYLGIKKFKIKSGRQFQ
EEDYNQFSRVILLEEKLAQRLFQTNEAALNKVVTVKNKSYLVVGVYSDPEAGSGLYGSNSDGNAILTNTQLASEFGA
KEAENIYFHLNDVSQSNRIGKEIGKRLTDISHAKDGYYDNFDMTSIVKSINTQVGIMTGVIGAIAAISLLVGGIGVM
NIMLVSVTERTREIGLRKALGATRRKILAQFLIESMVLTILGGLIGLLLAYGGTMLIANAQDKITPSVSLNVAIGSL
IFSAFIGIIFGLLPANKASKLNPIDALRYE

SeqID 321
MQYSEIMIRYGELSTKKKNRMRFINKLKNNMEHVLSIYPDVSVKTDRDRGHVYLNGTDYHEVAESLKEIFGIQAFSP
SFKVEKNVDTLVKAVQEIMTSVYKDGMTFKITAKRSDHSFELDSRALNHTLGDAVFSVLPNIKAQMKQPDINLKVEI
RDEAAYISYENIRGAGGLPVGTSGKGMLMLSGGIDSPVAGYLALKRGVDIEAVHFASPPYTSPGALKKAHDLTRKLT
KFGGNIQFIEVPFTEIQEEIKEKAPEAYLMTLTRRFMMRITDRIRENRNGLVIINGESLGQVASQTLESMQAINAVT
ATPIIRPVVTMDKLEIIDIAQKIDTFDISIQPFEDCCTIFAPDRPKTNPKIKNTEQYEKRMDVEGLVERAVAGIMVT
TIQPQADSDDVDDLIDDLL

SeqID 322
MARVKGGVVSRKRRKRVLKLAKGYYGAKHILFRTAKEQVMNSYYYAYRDRRQKKRDFRKLWITRINAAARMNGLSYS
QLMHGLKLAEIEVNRKMLADLAVNDAAAFTALADAAKAKLGK

SeqID 323
MFKASKKLVQKNKSNHFWLVFFITLILFLIGCYASLRFGAINFKTSDLITVLKNPLKNSNAQDVIFDIRLPRIIAAI
LVGAAMSQAGAIMQGVTRNAIADPGLLGINAGAGLALVVAYAFLGSMHYSTILIVCLLGSVISYLLVFTLSYTKQKG
YHQLRLILAGAMISTLFTSVGQVVTLYFKLNRTVIGWQAGGLSQINWKMLIIIAPIIILGLLISQLLAHQLTILSLN
ESVAKALGQKTQLMTAFLLLIVLFLSASSVALIGTVSFIGLIIPHFIKLFIPKDYRLLLPLIGFSGATFMIWVDLSS
RIINPPSETPISSIISIVGLPCFLWLIRKGKNL

SeqID 324
MIKRPIHLSHDFLAEVIDKEAITLDATMGNGNDTVFLAKSSKKVYAFDIQEEAIAKTKAKLTEQGISNAELILDGHE
NLEQYVHTPLRAAIFNLGYLPSADKTVITKPHTTIKAIKNVLDILEVGGRLSLMVYYGHDGGKSEKDAVIAFVEQLP
QNNFATMLYQPLNQVNTPPFLIMVEKLQSYENEV

SeqID 325
MRVHITSIYGQSPRSIALISQKLVKDVGRQLGYDEMGIYFYNDHAETHGERSTRMDGIIAGLGRGDIVVFQVPTWNS
TEFDELFLDKLQAYGARIITFVHDIVPLMFESNFYLLDRVIDMYNRSDVVILPTKAMHDYLIEKGMTTSKVLYQEVW
DHPVNIDLPRPECQKVLSFAGDIQRFPFVNDWKENIPLIYYGDGSRLNSEANVHAQGWKDDVELMLSLSKRGGFGLC
WSEDREELVERRYSRMNASYKLSTFLAAGLPIIANHDISSRDFIKQHGLGFTVETLEEAVEKINNMEKETYDSYVEN
VEKIATLLRNGYITKKLLIDAVHMLYR

SeqID 326
MSQKTFGKQLTVVDTKSRVKMHKSGKNWVRTVMSHFNLFKAIKGRATVEADVCIQDVEKEDRLSSGNLTYLKGILAA
GALVGGASLTSRVYADETPVVQEQSSSVPTLAEQTEVTVKTTTVQNHQDGTVSKNIIDSNSVSMSESASTSTSESVS
MSMSGSTLTSVSESVSTSALTSASESISTSASESVSKSTSISEVSNILETQASLTDKGRESFSANQIVTESSLVTDA
GKNASVSSLIEITKPKSELQTSKMSNESLITPEKSQVMIASDKTGNESLTPTIRLKSVIQPRSMNLMTLSSEMDLIP
LEEVSDTEMLGKDVSSELQKVNIALKDNTLSEPGTVKLDSSENLVLNFAFSIASVNEGDVFTVKLSDNLDTQGIGTI
LKVQDIMDETGQLLATGSYSPLTHNITYTWTRYASTLNNIKARVNMPVWPDQRIISKTTSDKQCFTATLNNQVASIE
ERVQYNSPSVTEHTNVKTNVRSRIMKLDDERQTETYITQINPEGKEMYFASGLGNLYTIIGSDGTSGSPVNLLNAEV
KILKTNSKNLTDSMDQNYDSPEFEDVTSQYSYTNDGSKITIDWKTNSISSTTSYVVLVKIPKQSGVLYSTVSDINQT
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D

SeqID 327
MIDRKDTNRFKLVSKYSPSGDQPQAIETLVDNIEGGEKAQILKGATGTGKTYTMSQVIAQVNKPTLVIAHNKTLAGQ
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SPKEYADSVVSLRPGQEISRDQLLNNLVDIQFERNDIDFQRGKFRVRGDVVEVFPASRDEHAFRIEFFGDEIDRIRE
IESLTGRVLGEVEHLAIFPATHFMTNDEHMEEAISKIQAEMENQVELFEKEGKLIEAQRIRQRTEYDIEMLREMGYT
NGVENYSRHMDGRSEGEPPFTLLDFFPEDFLIMIDESHMTMGQIKGMYNGDRSRKEMLVNYGFRLPSALDNRPLRRE
EFESHVHQIVYVSATPGDYEMEQTDTVVEQIIRPTGLLDPEVEVRPSMGQMDDLLGEINLRTEKGERTFITTLTKRM
AEDLTDYLKEMGVKVKYMHSDIKTLERTEIIRDLRLGVFDVLIGINLLREGIDVPEVSLVAILDADKEGFLRNERGL
IQTIGRAARNSNGHVIMYADKITDSMQRAMDETARRRRLQMDYNEKHGIVPQTIKKEIRDLIAITKSNDSDKPEKVV
DYSSLSKKERQAEIKALQQQMQEAAELLDFELAAQIRDVILELKAID

SeqID 328
MKHKLKAFTLALLSIFFVFGGKVSAETVNIVSDTAYAPFEFKDSDQTYKGIDVDIVNEVAKRAGWNVNMTYPGFDAA
VNAVQSGQADALMAGTTVTEARKKVFNFSDTYYDTSVILYTKNNNKVTNYKQLKGKVVGVKNGTAAQSFLEENKSKY
GYKVKTFDTSDLMNNSLDSGSIYAAMDDQPVVQFAINQGKAYAINMEGEAVGSFAFAVKKGSGHDNLIKEFNTAFAQ
MKSDGTYNDIMDKWLGKDATKTSGKATGNANEKATPVKPSYKIVSDSSFAPFEYQNGKGKYTGFDMELIKKIAKQQG
FKLDISNPGFDAALNAVQSGQADGVIAGATITEARQKIFDFSDPYYTSSVILAVKKGSNVKSYQDLKGKTVGAKNGT
ASYTWLSDHADKYNYHVKAFDEASTMYDSMNSGSIDALMDDEAVLAYAINQGRKFETPIKGEKSGDIGFAVKKGANP
ELIKMFNNGLASLKKSGEYDKLVKKYLSTASTSSNDKAAKPVDESTILGLISNNYKQLLSGIGTTLSLTLISFAIAM
VIGIIFGMMSVSPSNTLRTISMIFVDIVRGIPLMIVAAFIFWGIPNLIESITGHQSPINDFVAATIALSLNGGAYIA
EIVRGGIEAVPSGQMEASRSLGISYGKTMQKVILPQAVRLMLPNFINQFVISLKDTTIVSAIGLVELFQTGKIIIAR
NYQSFRMYAILAIMYLVIITLLTRLAKRLEKRLK

SeqID 329
MGDKPISFRDKDGNFVSAADVWNAEKLEELFNTLNPNRKLRLEREKLAKEKG

SeqID 330
METYTLSNTLNIPKIGFGTWQLTEGEEAYKAVTHALKVGYTHIDTAQIYGNEHSVGRAIRDSGLARESIFLTTKIWN
DKHDYHLAKASIDESLQKLGVDYIDLLLIHWPNPKALRENDAWKAGNAGTWKAMEEAYKEGKVKAIGVSNFMKHHLE
ALFETAEIKPMVNQIILAPGCAQEDLVRFCKGNDILLEAYSPFGTGAIFENESIKAIAEKYGKSVAQVALRWSLDNG
FLPLPKSATPKNIEANLDIFDFQLNEDDITTLIQLDSGIKPKDPDNVSF

SeqID 331
MVKGQGNVVAQNKKAHHDYTIVETIEAGIVLTGTEIKSVRAARITLKDGYAQIKNGEAWLINVHITPYDQGNIWNQD
PDRTRKLLLKKREIEKISNELKGTGMTLVPLKVYLKDGFAKVLLGLAKGKHDYDKRESIKRREQNRDIARQLKNYNS
R

SeqID 332
MDHFTKLWQDFSKLPNVVAIALGGSRSGDSFDQSSDYDLYVYCAATPDITSRKRILNKHCHYIELNNHYWELEDNGT
LNDGTDIDILYRNIDNFLSDLEDVVEHHNSRIGYTTCFWHNLINCQILYDPENQLQSLKERFEVSYPSQLQKQIIIQ
NRNLLTGKLPSYDKQIIKALKRQDFVSTHHRTTAFLDSYFDIIFALNKLTHPGEKRMISYAKKNATLLPKHFEENII
KLCHHNSNEHTVKETLNDIIMHLDVMLKENFQHFIG

SeqID 333
MKKIIYLGLACVSILTLSGCESIERSLKGDRYVDQKLAENSSKEATEQLNKKTKQALKADKKAFPQLDKAVAKNEAQ
VLIKTSKGDINIKLFPKYAPLAVENFLTHAKEGYYNGLSFHRVIKDFMIQSGDPNGDGTGGKSIWNSKDKKKDSGNG
FVNEISPYLYNIRGSLAMANAGADTNGSQFFINQSQQDHSKQLSDKKVPKVIIKAYSEGGNPSLDGGYTVFGQVISG
METVDKIASVEVTKSDQPKEKITITSIKVIKDYKFK

SeqID 334
MKIGIIAAMEEELKLLVENLEDKSQETVLSNVYYSGRYGEHELVLVQSGVGKVMSAMSVAILVESFKVDAIINTGSA
GAVATGLNVGDVVVADTLVYHDVDLTAFGYDYGQMSMQPLYFHSDKTFVSTFEAVLSKEEMTSKVGLIATGDSFIAG
QEKIDVIKGHFPQVLAVEMEGAAIAQAAQATGKPFVVVRAMSDTAAHDANITFDEFIIEAGKRSAQVLMAFLKAL

SeqID 335

MEKRLSLGALVLASTVLLAACGNVGGGASSTGTKIGKDIKVGYNWELSGNVSSYGNSMKNGADLAVKEINAAGGVGG
KKLKVLSQDNKSENAEAATVATNLVTKGANVIIGPATSGAAASSTPKVNAAAVPMIAPAATQDNLVYGSDGKTLNQY
FFRATFVDNYQGKLLSQYATDNLKAKKVVLFYDNSSDYSKGVAKSFKESYSGKIVDSMTFSAGDTDFQASLTKLKGK
EYDAIVMPGYYTETGLIVKQARDLGISKPVLGPDGFDSPKFVQSATPVGASNVYYLTGFTTQGSTKAKAFHDHYVKA
YGEEPSMFSALSYDAVYMAAKSAKGAKTSIDLKKALAKLKDFKGVTGKMSIDKNHNVVKSAYVVKLDDGKTSSVNII
SAK

SeqID 336
MENHNSIKQTYGLMTTIAMIVGVVIGSGIYFKVDDILKFTGGDVFLGMVILVLGSFSIVFGSLSISELAIRTSESGG
IFSYYEKYVSPALAATLGLFASFLYLPTLTAIVSWVAAFYTLGESSSLESQIILAAVYILALSLMNIFAKRIAGGFQ
SLTTFVKMIPLVLIALIGAFWSDKAPQLPQHLTAIQPSNVGWSWVSGLVPLYFAYDGWTIFVSIAPEVKNPKKNLPL
AFVIGPALILLSYLAFFYGLTQILGASFIMTTGNDAINYAANIIFGPSVGRLLSFIVILSVLGVANGLLLGTMRLPQ
AFAERGWIKSERMANINLKYQMSLPASLTVTAVAIFWLFVHFMVTKFNLLPGSDISEIAVVFNNTSLIILYVLVLSL
YLKKDIKNKFTGLVSPILAILGGLILFIGSLLSNFFTVLIFQCFCLLFCLICHYIYQKNNPKTHE

SeqID 337
LTEFNDDQHSNHDQKSFKEQILAELEEANRLRKLREEELYQKEQEAKEAARRTAQLMADYEAQRLKDEQEARAKALE
TKQRLEEQEKARIEAKLLAEAAREEERRQAEQALASQEEQVINQGMEPSRELDSGSKSSEFRTTENVPDIDLKADKT
DVATAVPNQETEEIFLVRATDIPTEGENVKLGETSELEPVAKEPIRVEDLSKEEEDIALSAKNKHNKRERRQKADNV
AKRIARILISIIILVLLLTAFVGYRFVDSAIKPVDSNSNKFVQVEIPIGSGNKLIGQILEKAGVIKSATVFNYYSKF
KNYSNFQSGYYNLKKSMTLDQIAAELEKGGTAEPTKPALGKILITEGYTIKQIAKAIESNKIDTKTTSTPYKADDFL
KLVQDETFIKKMVAKYPNLLGSLPDKSKAIYQLEGYLFPATYNYYKDTTLEGLVEDMISTMNTKMAPYYNTIKAKNM
SVNDVLTLSSLVEKEGSTDEDRRKIASVFYNRLSAGQALQSNIAILYAMGKLGDKTSLAEDAQINTSIKSPYNIYTN
TGLMPGPVDSPSISAIEATIKPASTDYLYFVADVKTGNVYYAKDFETHKANVEKYINSQIN

SeqID 338
MSRMIPGRIRNQGIELYEQGLVSLISQEGNLLKAKVGDCQIEYSLVTEETKCSCDFFARKGYCQHLAALEHFLKNDP
EGKAILSKVQVQQESQQETKKKTSFGSVFLDSLIINEDDTIKYQLSAQGEQNPYANDIWWTLKIRRLPDDRSYVIRD
IKAFLNTVRKEAYYQIGKQYFETLSLIQFDETSQELIEFLWRLIPSHSSKIDLEFILPNQGRHLSLTRGFFEEGVTL
MNALENFSFESDFHQFNHLYFKELEGEDHLYQFKVIVHRQSIELEIKEKDLKPLFANSYLFYRDTFYHLNLKQEKMV
TAIRSLPIEGDLAKHIHFDLDDQDKLAAHLLDFKEIGLVDAPRSFSIHDFKVNFEFDINSQNEILLQMVFDYGNDLT
VHNRQELEQLTFASHFKHEEKIFKLLERYGFAPHFSTSHPAYSAQELYDFYTYMLPQFKKMGTVSLSAKLESYRLIE
RPQIDIEAKGSLLDISFDFSDLLENDVDQALVALFDNNPYFVNKSGQLVIFDEETKKVSATLQGLRARRAKNGHIEL
DNIAAFQLSELFANQDNVSFSQHFYQLIEDLRHPEKFKIPGLSVSASLRDYQLTGVRWLSMLDHYGFAGILADDMGL
GKTLQTISFLSTKLTRDSRVLILSPSSLIYNWQDEFHKFAPDVDVAVAYGSKIRRDEIIAERHQVIITSYSSFRQDF
ETYSEGNYDYLILDEAQVMKNAQTKIAHSLRSFEVKNCFALSGTPIENKLLEIWSIFQIILPGLLPGKKEFLKLNPK
QVARYIKPFVMRRRKEEVLPELPDLIEMNYPNEMTDSQKVIYLAQLRQIQESIQHSSDADLNRRKIEILSGITRLRQ
ICDTPRLFMDYDGESGKLESLHQLLTQIKENGHRALIFSQFRGMLDIAEREMVAMGLTTYKITGSTPANERHEMTRA
FNAGSKDAFLISLKAGGVGLNLTGADTVVLIDLWWNPAVEMQAISRAHRLGQKENVEVYRLITRGTIEEKILEMQET
KKHLVTTVLDGNETHASMSVDDIREILGVSK

SeqID 339
VLLTEIKKSPEGLYFDKKIDIKESLMERHSEIMDISDIQVSGHVVYEDGLYLLDYNMAYDITLPSSRSMKPVVLSEK
QTINEVFIEAENVSTKKELVDQDLVLILEEDDINLEESVIDNILLNIPLRVLAADEVGVEADLSGKNWSLMTEKQYE
EKQAKEKEKSNPFAALEGMFDSD

SeqID 340
MEVKTAIEWMHTFNQKIQSNKDYLSELDTPIGDGDHGGNMARGMTAVIENLDNNEFSSAADVFKTVSMQLLSKVGGA
SGPLYGSAFMGITKAEQSESTISEALGAGLEMIQKRGKAELNEKTMVDVWHGVIEAIEKNELTEDRIDSLVDATKGM
KATKGRASYVGERSLGHIDPGSFSSGLLFKALLEVGGV

SeqID 341
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IIRRKKGQHKSTFEKIQKDGYVRVRIDGDIHDVTEVPELSKSKMHNIDIVVDRLINKEGIRSRLFDSVEAALRLSDG
YVVIDTMDGNELLFSEHYSCPECGFTVPELEPRLFSFNAPFGSCTTCDGLGIKLEVDIDLVIPDKSKTLREGALVPW
NPISSNYYPTMLEQAMTQFDVDMDTPFEKLSKAEQDLALYGSGEREFHFHYINDFGGERNIDLPFEGVVNNINRRYH
ETNSDYTRNVMREYMNELKCNTCHGYRLNDQALCVRVGGEEGLNIGQVSDLSIADHLELLETLRLSSNEQLIARPII
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GNTLIVVEHDEDTMMAADWLIDVGPGAGAFGGEIVASGTPKQVAKNTKSITGQYLSGKKVIPVPSERRVGNGRFLEI
KGAAENNLQNLDVKFPLGKFIAVTGVSGSGKSTLINSILKKAVAQKLNRNSDKPGKYVSLEGIEYVDRLIDIDQSPI
GRTPRSNPATYTGVFDDIRDLFAQTNEAKIRGYKKGRFSFNVKGGRCESCSGDGIIKIEMHFLPDVYVPCEVCHGTR
YNSETLEVHYKEKNIAQILDMTVNDAVTFFAAIPKIARKLQTIKDVGLGYVTLGQPATTLSGGEAQRMKLASELHKR
STGKSLYILDEPTTGLHADDIARLLKVLDRFVDDGNTVLVIEHNLDVIKTADHIIDLGPEGGIGGGQIVAIGTPEEV
AENPKSYTGYYLKEKLAR

SeqID 342
MWPEDRIASFRRTLLGWYDQEKRDLPWRRTTNPYYIWVSEIMLQQTQVNTVIPYYKRFLEWFPQIKDLADAPEEQLL
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NYDIGDPKNRKIFQAIMEILIDPDRPGDFNQALMDLGTDIESAKTPRPDESPIRFFNAAYLNGTYSKYPIKNPKKKP
KPMRIQAFVIRNQNGQYLLEKNTKGRLLGGFWSFPIIETSPLSQQLDLFDDNQSNPIIWQTQNETFQREYQLKPQWT
DNHFPNIKHTFSHQKWTIELIEGVVKATDLPNAPHLKWAAIEDFSLYPFATPQKKMLETYLKQKNA

SeqID 343
LIEITWTVKYITEFIATAFLIILGNGAVANVDLKGTKGNNSGWIIIAIGYGLGVMMPALMFGNVSGNHINPAFTLGL
AFSGLFPWAHVGQYILAQILGAMFGQLVVVMVYQPYFVKTENPNHVLGSFSTISALDDGQKSSRKAAYINGFLNEFV
GSFVLFFGALALTKNYFGVELVGKLVQAGYDQTTAATRISPYVTGSLAVAHLGIGFLVMTLVASLGGPTGPALNPAR
DLGPRIVHRLLPKQILGQAKEDSKWWYAWVPVLAPIVASILAVALFKLLYL

SeqID 344
MSSYWNNYPELKKNIDETNQLIQERIQVRNKDIEAALSQLTAAGGKQLRPAFFYLFSQLGNKENQDTQQLKKIAASL
EILHVATLIHDDVIDDSPLRRGNMTIQSKFGKDIAVYTGDLLFTVFFDLILESMADTPFMRINAKSMRKILMGELDQ
MHLRYNQQQGIHHYLRAISGKTAELFKLASKEGAYFGGAEKEVVRLAGHIGFNIGMTFQILDDILDYTADKKTFNKP
VLEDLTQGVYSLPLLLAIEENPDIFKPILDKKTDMATEDMEKIAYLVVSHRGVDKARHLARKFTEKAISDINKLPQN
SAKKQLLQLTNYLLKRKI

SeqID 345
MFKIPLFKELKTDQWVKPFFKQYKVSLVIALFLGFMTFFSASALMFNSGYLISKSASLPSNILLVYVPIVLTRAFGI
GRPVFRYIERLTSHNWVLRMTSQLRLKLYHSLESNAIFMKRDFRLGDVMGLLAEDINYLQNLYLRTIFPTIIAWLLY
SFIIIATGFFSLWFALMMLLYLAIMIFLFPLWSILANGARQTREKELKNHLYTDLTDNVLGISDWIFSQRGQEYVAL
HERSESELMAIQKKIRSFNNRRALIVELVFGFLAILVIIWASNQFIGHRGGEANWIAAFVLTVFPLSEAFAGLSAAA
QETNKYSDSIHRLNELSETYFETTQNQLPNKPYDFSVKNLSFQYKPQEKWVLHHLDLDIKEGEKIAILGRSGSGKST
LASLLRGDLKASQGKITLGGADVSIVGDCISNYIGVIQQAPYLFNTTLLNNIRIGNQDASEEDVWKVLERVGLKEMV
TDLSDGLYTMVDEAGLRFSGGERHRIALARILLKDVPIVILDEPTVGLDPITEQALLRVFMKELEGKTLVWITHHLK
GIEHADRILFIENGQLELEGSPQELSQSSQRYRQLKAADDGDL

SeqID 346
MKKLITEKKVNNVSTVNYLKLGLVSAMFAGGAFVALGSTQGVSASTFTAPQATHPKAERQLTDSEIYERAQKQVLPK
YIQGSLSGILNQHSTLYKQQNAAVTPQVSSPKAERQLTDSEIYERAQKQVLPKYIQGSLSGILNQHSTLNA

SeqID 347
MEENMNIKQLKSKTMLGTVALVSAFSFASTNADANTYNYAVDVDYLASAEEIAQAHPASNTFPLGQCTWGVKEMATW
AGNWWGNGGDWAASAASAGYTVGTQPRVGSIVCWTDGSYGHVAYVTAVDPVTNKIQVLESNYAGHQWIDNYRGWFDP
QNTATPGVVSYIYPN

SeqID 348
MKINQMKKDELFEGFYLIKKAEVRKTRAGKDFIAFTFRDDTGEISGNMWDAQTYNVEEFVAGKIVHMKGRREVYNGT
PQVNQITLRNIKDGEPNDPRDFKEKPPINVDNVREYMEQMLFKIENATWQRVVRALYRKYNKEFFTYPAAKTNHHAF
ESGLAYHTATMVRLADSIGDIYPELNKSLMFAGIMLHDLAKVIELSGPDNTEYTIRGNLIGHISLIDEELTKILAEL
NIDDTKEEVTVLRHVILSHHGQLEYGSPVRPRIMEAEIIHMIDNIDANMMMMTTALNRVNEGEMTNRIFAMDNRSFY
KPNIK

SeqID 349
MLVDKKWRFEDSASYFACPKCQNPLIKESNSLKCSDNHCFDLSKFGYVNLLGGKKIDEHYDKKSFENRQLVLENGYY
NHILEAISKVLENNSQFHSVLDIGCGEGFYSRQLVNKYEKTFLAFDISKDSIQLAAKSDQSRLVKWFVSDLANLPIQ
DSSIDIILDIFSPANYKEFRRVLSDDGILVKVVPVAEHVQELREKASQYLKQKDYSNQKILDHFRENFEIISEQKVV
QSYNCSQQERQAFIDMTPLLFSVDKTTIDWASISEITVGALIVIGKKRSVSK

SeqID 350
MCLICQRIEMIERNENPYFVKEYETGYLVLGDHQYFQGYCLFLSKKHVTELHELPRDWRNQYLSEMADASEIVAKAF
RADKMNIESLGNGDAHLHFHLFPRKTGDLRNYGHNGKGPVWWYPFEKMYADSVRATGAEIEKLKEKLLDVLG

SeqID 351
MFSDLRKKFVFLTMSILIVVVLFLFAVSNRYNQYWDEYDAYRIVKLVAKNDYLGIPGDEPIALVTIDNQKMVKIQSN
NTDLTNDVIEKSSLKLLEQGKKSRKWKSFIYSIKEYKDKTYTIAIMDLASYEVPYARRFLILVFTIFGFCLLAAVSL
YLSRFIVGPVETEMTREKQFVSDASHELKTPIAAIRANVQVLEQQIPGNRYLDHVVSETKRMEFLIEDLLNLSRLDE
KRSKVNFKKLNLSVLCQEVLLTYESLAYEEEKCLNDTIEDDVWIVGEESQIKQILIILLDNAIRHSLSKSEIQFSLK
QARRKAILTISNPSAIYSKEVMDNLFERFYQAKDDHADSLSFGLGLSIAKAIVERHKGRIRAYQEKDQLRLEVQLPI
DGFWTNTMIN

SeqID 352
LIILDKKSYDLLFYLLKLEEPETVMAIANALNQSRRKVYYHLEKINDALPSDVPQIVSYPRVGILLTEKQKAACRLL
LDEVTDYSYVMKSSERLQLSLVSIVVAKDRITIDRLMQLNDVSRNTILNDLNELRSELAEKEYNLQLQSTKCRGYFL
DGHPLSIIQYLYKLLDDIYHNGSSSFIDLFNHKLSQAFGASTYFSKEVLDYFHHYLFISQRSLGKKINSQDGQFMIQ
ILPFILMAYRKMRLSPEVQTSLNSDFSLVWQRKEYEIAKELADELEENFQLSLDEIEVGLVAMLMLSFRKDRDNHLE
SQDYDDMRATLTSFLKELEERYHLHFVHKKDLLRQLLTHCKALLYRKRYGIFSVNPLTEHIKDKYEELFAITSSSVK
LLEKAWQIKLTDDDVAYLTIHLGGELRNSQQSPNKLKLVIVSDEGIAIQKLLLKQCQRYLTNSDIEAVFTTEQYQSV
SDLMHVDMVVSTSDALESRFPMLVVHPVLTDDDIIRLIRFSKKGNCANSNQFTNELEKTIAQYVKEDSERYVLKSKI
EKLIHQELLQDVLPLQSTVC

SeqID 353
MKYFLDTADVSEIRRLNRLGIVDGVTTNPTIISREGRDFKEVINEICQIVDGPVSAEVTGLTCDEMVTEAREIAKWS
PNVVVKIPMTEEGLAAVSQLSKEGIKTNVTLIFTVAQGLSAMKAGATFISPFVGRLEDIGTDAYALIRDLRHIIDFY
GFQSEIIAASIRGLAHVEGVAKCGAHIATIPDKTFASLFTHPLTDKGIETFLKDWDSFKKK

SeqID 354
MSHYSIKLQEVFRLAQFQAARYESHYLESWHLLLAMVLVHDSVAGLTFAEYESEVAIEEYEAATILALGRAPKEEIT
NYQFLEQSPALKKILKLAENISIVVGAEDVGTEHVLLAMLVNKDLLATRILELVGFRGQDDGESVRMVDLRKALERH
AGFTKDDIKAIYELRNPKKAKSGASFSDMMKPPSTAGDLADFTRDLSQMAVDGEIEPVIGRDKEISRMVQVLSRKTK
NNPVLVGDAGVGKTALAYGLAQRIANGNIPYELRDMRVLELDMMSVVAGTRFRGDFEERMNQIIADIEEDGHIILFI
DELHTIMGSGSGIDSTLDAANILKPALARGTLRTVGATTQEEYQKHIEKDAALSRRFAKVLVEEPNLEDAYEILLGL
KPAYEAFHNVTISDEAVMTAVKVAHRYLTSKNLPDSAIDLLDEASATVQMMIKKNAPSLLTEVDQAILDDDMKSASK
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TGKRPIGSFMFLGPTGVGKTELAKALAEVLFDDESALIRFDMSEYMEKFAASHLNGAPPGYVGYDEGGELTEKVRNK
PYSVLLFDEVEKAHPDIFNVLLQVLDDGVLTDSRGRKVDFSNTIIIMTSNLGATALRDDKTVGFGAKDISHDYTAMQ
KRIMEELKKAYRPEFINRIDEKVVFHSLSQDNMREVVKIMVKPLILALKDKGMDLKFQPSALKHLAEDGYDIEMGAR
PLRRTIQTQVEDHLSELLLANQVKEGQVIKIGVSKGKLKFDIAKS

SeqID 355
MVLDKEIKAQLAQYLDLLESDIVLQADLGDNDNSQKVKDFLDEIVAMSDRISLESTHLKRQPSFGIAKKGHESRVIF
SGLPMGHEFTSFILALLQVSGRAPKVDEDIIKRIKGIEKTINLETYVSLTCHNCPDVVQAFNIMAVLNPNITHTMIE
GGMYQDEVKSKGIMSVPTVYKDQEEFTSGRATIEQLLEQLDGPLDAEAFADKGVYDVLVIGGGPAGNSAAIYAARKG
LKTGILAETFGGQVIETVGIENMIGTLYTEGPKLMAQIEEHTKSYDIDIIKSQLATGIEKKELVEVTLANGAILQAK
TAILALGAKWRNINVPGEEEFRNKGVTYCPHCDGPLFEGKDVAVIGGGNSGMEAALDLAGVTKHVTVLEFLPELKAD
QVLQERAAKTDNLTILKNVATKDIVGEDHVTGLNYTDRDTNEEKHIDLEGVFVQIGLVPSTSWLKDSGIELNERQEI
VVDKFGSTNIPGIFAAGDCTDAAYKQIIISMGSGATAAIGAFDYLIRQ

SeqID 356
MIKYQDDFYQAVNGEWAKTAVIPDDKPRTGGFSDLADDIEALMLSTTDKWLADENKPSDTILNHFIAFHKMTADYQK
REEVGVSPVLPLIEEYKGLQSFSEFASKVAEYELEGKPNEFPFGVAPDFMNAQLNVLWAEAPGIILPDTTYYSEDNE
KGKELLAFWRKSQEDLLPLFGLSEQEIKDILDKVLALDAKLAQYVLSREESSEYVKLYHPYNWEDFTKLAPELPLDA
IFQKILGQKPDKVIVPEERFWTEFASDYYSESNWELLKADLILSAANAYNAYLTDDIRIKSGVYSRALSGTPQAMDK
KKAAYYLASGPYNQALGLWYAGEKFSPEAKADVEHKIATMIDVYKSRLEKADWLAQSTREKAIMKLNVITPHIGYPE
KLPETYTKKIIDPKLSLVENATNLDKISIAYGWSKWNKPVDRSEWHMPAHMVNAYYDPQQNQIVFPAAILQEPFYAL
EQSSSANYGGIGAVIAHEISHAFDTNGASFDEHGSLNNWWTDEDFEAFKKLTDKVVEQFDGLESYGAKVNGKLTVSE
NVADLGGVACALEAAQRESDFSARDFFINFATIWRMKARDEYMQMLASVDVHAPAQWRTNITVTNFEEFHKEFDVKD
GDNMWRPVEKRVIIW

SeqID 357
MSKILFFGEPLIRITPKENDYFADSISTKLFYGGSEVNTARALQGFGQDTKLLSALPNNPIGNSFLQFLKAQGIDTH
SIQWVGERVGLYFLEDSFACRKGEVVYDRDHSSLHDFRINQIDFDQLFEGVSLFHFSGITLSLDESIQEITLLLLKE
AKKREITISLDLNFRSKLISPKNAKILFSQFATFADICFGIEPLMVDSQDTTFFNRDEATIEDVKERMISLINHFDF
QVIFHTKRLQDEWGRNHYQAYIANRKQEFVTSKEITTAVNQRIGSGDAFVAGALYQLLQHSDSKTVIDFAVASASLK
CALEGDNMFETVTAVNKVLNQSKDIIR

SeqID 358
MKSRKKDKLVLRLTTTLLVFGLGGVWFYNYKNDNVEPTVTSASDQTTTFIQTISPTAIEISKTYDLYASVLLAQAIL
ESSSGQSDLSKAPNYNLFGIKGEYKGKSVQMPTLEDDGKGNMTQIQAPFRAYPNYSASLYDYAELVSSQKYASVWKS
NTSSYKDATAALTGLYATDTAYASKLNQIIETYSLDAYDK

SeqID 359
MADVKVVNNEDSRGQKQDLKAKLFHIKIGSVPLPVYVCLALLILLAGFLQKLPVNMLGGFAVILTMGWFLGTIGASI
PGFKNFGGPAILSLLVPSILVFFNLINKNVLESTNMLMKQANFLYFYIACLVSGSILGMNRKMLIQGLLRMIFPMLL
GMVCAMMVGTFVGVILGLEWRHTLFYIVTPVLAGGIGEGILPLSLGYSSITGVASEQLVAQLIPATIIGNFFAILCT
ALLNRLGEKKPHLSGQGQLVRLNKGEDMSDIIADHSGPIDVKKMGGGVLTACSLFIFGHLLQQLTGFPGPVLMIVAA

AILKYINVIPRETQNGAKQLYKFISGNFTFPLMAGLGLLYIPLKDVVATLSIQYFIVVISVVFTVISVGFFVSRFLN
MNPVEAGIISACQSGMGGTGDVAILSTADRMNLMPFAQVATRLGGAITVITMTAILRMLF

SeqID 360
RGKKAGKYTTSDGYIFDAKDIKKDTGTGYVIPHMTHEHWVPKKDLSESELKAAQEFLSGKSEANQDKPKTGKTAQEI
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KRPKVEGWGKDAEIYKEKDSNKADKPSPAPTDNKSTSNSSDKNLSAAEVFKQAKPEKIVPLDKIAAHMAYAVGFEDD
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INGSQVQVTKKDGSKVLVDIKTLTEVK

SeqID 361
MNRKKTVIISALSVALFGTGVGAYQLGSYNAQKSDNSVSYVKTDKSDSKAQATAVNKTPDQISKEEGISAEQIVVKI
TDDGYVTSHGDHYHYYNGKVPYDAIISEELIMKDPSYVFNKADVINEVKDGYIIKVNGKYYLYLKEGSKRTNVRTKE
QIQKQREEWSKGGSKGESGKHSSAKTQALSASVREAKASGRYTTDDGYVFSPTDVIDDMGDAFLVPHGDHFHYIPKA
DLSPSELSAAQAYWNRKTGRSGNSSKPSNSSSYIHASAPSGNVSTGRHANAPISIPRVTHANHWSKPAGNHATAPKH
HAPTTKPINKDSALDKMLKRLYAQPLYARHVESDGLVYDPAQVNAFTAIGVSIPHGNHFHFIHYKDMSPLELEATRM
VAEHRGHHIDALGKKDSTEKPKHISHEPNKEPHTEEEHHAVTPKDQRKGKPNSQIVYSAQEIEEAKKLVNTQHLMVT
FLMPKILKKIQVQVMSFHI

SeqID 362
MAKFGFLSVLEEELDKHLQYDFAMDWDKKNHTVEVTFILEAQNSSAIETVDDQGETSSEDIVFEDYVLFYNPVKSRF
DAEDYLVTIPYEPKKGLSREFLAYFAETLNEVATEGLSDLMDFLTDDSIEEFGLSWDTDAFENGRAELKETEFYPYP
RY

SeqID 363
MNTKQRFSIRKYKLGAVSVLLGTLFFLGGITNVAADSVINKPSDIAVEQQVKDSPTSIANETPTNNTSSALASTAQD
NLVTKANNSPTETQPVAESHSQATETFSPVANQPVESTQEVSKTPLTKQNLAVKSTPAISKETPQNIDSNKIITVPK
VWNTGYKGEGTVVAIIDSGLDINHDALQLNDSTKAKYQNEQQMNAAKAKAGINYGKWYNNKVIFGHNYVDVNTELKE
VKSTSHGMHVTSIATANPSKKDTNELIYGVAPEAQVMFMRVFSDEKRGTGPALYVKAIEDAVKLGADSINLSLGGAN
GSLVNADDRLIKALEMARLAGVSVVIAAGNDGTFGSGASKPSALYPDYGLVGSPSTAREAISVASYNNTTLVNKVFN
IIGLENNRNLNNGLAAYADPKVSDKTFEVGKQYDYVFVGKGNDNDYKDKTLNGKIALIERGDITFTKKVVNAINHGA
VGAIIFNNKAGEANLTMSLDPEASAIPAIFTQKEFGDVLAKNNYKIVFNNIKNKQANPNAGVLSDFSSWGLTADGQL
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LAPQQLGTFTGKTIRIEPGQTQTITIDIDVSKYHDMLKKVMPNGYFLEGYVRFTDPVDGGEVLSIPYVGFKGEFQNL
EVLEKSIYKLVANKEKGFYFQPKQTNEVPGSEDYTALMTTSSEPIYSTDGTSPIQLKALGSYKSIDGKWILQLDQKG
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GTTTDGIPLEDGKYKYVLTYYSDVPGSKPQQMVFDITLDRQAPTLTTATYDKDRRIFKARPAVEHGESGIFREQVCY
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LVNVKVFSPELNSNVDIDFSYSVKDDKGNIIKKQHHGKDLNLLKLPFGTYTFDLFLYDEERANLISPKSVTVTISEK
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NLDDLLVSVKEDQVNLTKLTLINKAPLINALAEQTDIITQPVFYNAGTHLKNNYLANLEKAQTLIKNRVEQTSIDNA
IAALRESRQALNGKETDTSLLAKAILAETEIKGNYQFVNASPLSQSTYINQVQLAKNLLQKPNVTQSEVDKALENLD
IAKNQLNGHETDYSGLHHMIIKANVLKQTSSKYQNASQFAKENYNNLIKKAELLLSNRQATQAQVEELLNQIKATEQ
ELDGRDRVSSAENYSQSLNDNDSLNTTPINPPNQPQALIFKKGMTKESEVAQKRVLGVTSQTDNQKVKTNKLPKTGE
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SeqID 364
MNNNEKKVKYFLRKTAYGLASMSAAFAVCSGIVHADTSSGISASIPHKKQVNLGAVTLKNLISKYRGNDKAIAILLS
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VKSISSSKDSQLILKFVTQATQLNNAESTKAKQMAQNDVALIKNISPEVLEEYKEKIQRASTKSQVDEFVAEAKKVV
NSNKETLVNQANGKKQEIAKLENLSNDEMLRYNTAIDNVVKQYNEGKLNITAAMNALNSIKQAAQEVAQKNLQKQYA
KKIERISSKGLALSKKAKEIYEKHKSILPTPGYYADSVGTYLNRFRDKQTFGNRSVWTGQSGLDEAKKMLDEVKKLL
KELQDLTRGTKEDKKPDVKPEAKPEAKPNIQVPKQAPTEAAKPALSPEALTRLTTWYNQAKDLLKDDQVKDKYVDIL
AVQKAVDQAYDHVEEGKFITTDQANQLANKLRDALQSLELKDKKVAKPEAKPEAKPEAKPEAKPEAKPEAKPEAKPE
AKPDVKPEAKPDVKPEAKPEAKPEAKSEAKPEAKLEAKPEAKPATKKSVNTSGNLAAKKAIENKKYSKKLPSTGEAA
SPLLAIVSLIVMLSAGLITIVLKHKKN

SeqID 365
MAKDIKFSADARSAMVRGVDILADTVKVTLGPKGRNVVLEKAFGSPLITNDGVTIAKEIELEDHFENMGAKLVSEVA
SKTNDIAGDGTTTATVLTQAIVREGLKNVTAGANPIGIRRGIETAVSAAVEELKEIAQPVSGKEAIAQVAAVSSRSE
KVGEYISEAMGRVGNDGVITIEESRGMETELEVVEGMQFDRGYLSQYMVTDNEKMVSELENPYILITDKKISNIQEI
LPLLEEVLKTNRPLLIIADDVDGEALPTLVLNKIRGTFNVVAVKAPGFGDRRKAMLEDIAILTGGTVVTEDLGLDLK
DATMQVLGQSAKVTVDKDSTVIVEGAGDSSAIANRVAIIKSQMEATTSDFDREKLQERLAKLAGGVAVIKVGAATET

ELKEMKLRIEDALNATRAAVEEGIVSGGGTALVNVIEKVAALKLNGDEETGRNIVLRALEEPVRQIAYNAGYEGSVI
IERLKQSEIGTGFNAANGEWVDMVTTGIIDPVKVTRSALQNAASVASLILTTEAVVANKPEPEAPTAPAMDPSMMGG
F

SeqID 366
VVENLEKPIGVSYKNSPSMSKRTAIRMKKSSRFSILLYSVLSTLLAIANPLLTYFANGLQTQNLYTGLMMTKGQIPY
SDVFATGGFLYYVTIALSYLLGSSIWLLIVQFIAYYVSGIYFYKLVYYVAQSEIVSIGMTLIFYIMNIVLGFGGMYP
IQWALPFMLISLWFLIKFCVDNIVDEAFIFYGILAAFSLFIDPQTLIFWLCSFVLLTATNIKQKQSLRGFYQFLCVV
FGMILIAYTVGYFMFNLQIISSYIDKAIFYPFTYFARTNHSFLLSLAIQIVVLLGSGCLFGLWDFIQNRKKASYQIG
LNFIACIFIIYAIMAIFSRDFNLYHFLPALPFGLLLTSNKITILYQKVIDRRSHRRQYFSGKSLIVDLFVKKTYYLP
LLLVSLSIGLLVYNTYQNVTLSKERRDISHYLTTKIDRDGKIYVWDKVASIYSQTRLKSASQFVLPHINTAQKNNEK
ILKDELLQHGAKYFILNKNEKLPNELKSDIKKHYQEVPLSNITHFVLYRFK

SeqID 367
MAKPTISPGMQQYLDIKENYPDAFLLFRMGDFYELFYDDAVKAAQILEISLTSRNKNAEKPIPMAGVPYHSAQQYID
VLVELGYKVAIAEQMEDPKKAVGVVKREVVQVVTPGTVVESTKPDSANNFLVAIDSQDQQTFGLAYMDVSTGEFQAT
LLTDFESVRSEILNLKAREIVVGYQLTDEKNHLLTKQMNLLLSYEDERLNDIHLIDEQLTDLEISAAEKLLQYVHRT
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DTMPELESLINTAIAPEAQATITEGNIIKSGFDKQLDNYRTVMREGTGWIADIEAKERAASGIGTLKIDYNKKDGYY
FHVTNSNLSLVPEHFFRKATLKNSERYGTAELAKIEGEMLEAREQSSNLEYDIFMRVRAQVESYIKRLQELAKTIAT
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ALTVIMAQMGGFVSADEVDLPVFDAIFTRIGAADDLISGQSTFMVEMMEANQAVKRASDKSLILFDELGRGTATYDG
MALAQSIIEYIHDRVRAKTMFATHYHELTDLSEQLTRLVNVHVATLERDGEVTFLHKIESGPADKSYGIHVAKIAGL
PIDLLDRATDILSQLEADAVQLIVSPSQEAVTADLNEELDSEKQQGQLSLFEEPSNAGRVIEELEAIDIMNLTPMQA
MNAIFDLKKLL

SeqID 368
MLKLDLKTKIKEAILIAFGVALYTFGFVKFNMANHLAEGGISGVTLIIHALFGVNPALSSLLLNIPLFILGARILGK
KSLLLTIYGTVLMSFFMWFWQQIPVTVPLKNDMMLVAVAAGILAGTGSGLVFRYGATTGGADIIGRIVEEKSGIKLG
QTLLFIDAIVLTSSLVYINLQQMLYTLVASFVFSQVLTNVENGGYTVRGMIIITKESESAAATILHEINRGVTFLRG
QGAYSGREHDVLYVALNPSEVRDVKEIMADLDPDAFISVINVDEVISSDFKIRRRNYDK

SeqID 369
MKRSMYAGRVRSEHIGTSITLKGWVGRRRDLGGLIFIDLRDREGIMQLVINPEEVAASVMATAESLRSEFVIEVSGV
VTAREQANDNLPTGEVELKVQELSVLNTSKTTPFEIKDGIEANDDTRMRYRYLDLRRPEMLENFKLRAKVTHSIRNY
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FTQVDLETSFLSDQEIQDIVEGMIAKVMKDTKGLEVSLPFPRMAYDDAMNNYGSDKPDTRFDMLLQDLTEIVKEVDF
KVFSEASVVKAIVVKDKADKYSRKNIDKLTEIAKQYGAKGLAWLKYVDNTISGPVAKFLTAIEGRLTEALQLENNDL
ILFVADSLEVANETLGALRTRIAKELELIDYSKFNFLWVVDWPMFEWSEEEGRYMSAHHPFTLPTAETAHELEGDLA
KVRAVAYDIVLNGYELGGGSLRINQKDTQERMFKALGFSAESAQEQFGFLLEAMDYGFPPHGGLAIGLDRFVMLLAG
KDNIREVIAFPKNNKASDPMTQAPSLVSEQQLEELSLTVESYEN

SeqID 370
KEKGKLIKKKILENNTDIIPKIIEKSPQNLILTSNYNRVNIDKIKNIKNFDKGFELGFPLFEKGEILRKEGEITSAI
ELFDKARELGYFVPALYNSYAMAFRKIKNYDDEILILQEGIERFKKSTLSSNINPKTIDRWSTRISRAKDLKCK

SeqID 371
LNVKKHHLAYGAITLVALFSCILAVMVIFKSSQVTTESLSKADKVRVAKKSKMTKATSKSKVEDVKQAPKPSQASNE
APKSSSQSTEANSQQQVTASEEAAVEQAVVTENTPATSQAQQAYAVTETTYRPAQHQTSGQVLSNGNTAGAIGSAAA
AQMAAATGVPQSTWEHIIARESNGNPNVANASGASGLFQTMPGWGSTATVQDQVNSAIKAYRAQGLSAWGY

SeqID 372
MSNWDTKFLKKGFTFDDVLLIPAESHVLPNEVDMKTKLADNLTLNIPIITAAMDTVTDSKMAIAIARAGGLGIIHKN
MSIVDQAEEVRKVKRSENGVIIDPFFLTPDNTVSEAEELMQNYRISGVPIVETLENRKLVGIITNRDMRFISDYKQL
ISEHMTSQNLVTAPIGTDLETAERILHEHRIEKLPLVDDEGRLSGLITIKDIEKVIEFPKAAKDEFGRLLVAGAVGV
TSDTFERAEALFEAGADAIVIDTAHGHSAGVLRKIAEIRAHFPNRTLIAGNIATAEGARALYDAGVDVVKVGIGPGS
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SeqID 449
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SeqID 450
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SeqID 452
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SeqID 453
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SeqID 454
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SeqID 456
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SeqID 457
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SeqID 458
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SeqID 460
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SeqID 461
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SeqID 462
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SeqID 463
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ttgacgggag acgttaacca tcaatggaga atgctagtaa tctttttagt atcatcgatg   2280
atacttgctc ttcttattta tcgtaaacaa gaagattaa                          2319
```

SeqID 475

```
MLKKSHFLQIFTLCLALLTISGCQLTDTKKPGHTTIKVAAQSSTESSIMANIVTELIHHELGYNTTLISNLGSSTVT
HQALLRGDADIAATRYTGTDITGTLGLKAVKDTKEASKIVKTEFQKRYNQTWYPTYGFSDTYAFMVTKEFARQNKIT
KISDLKKLSTTMKAGVDSSWMNREGDGYTDFAKTYGFEFSHIYPMQIGLVYDAVESNKMQSVLGYSTDGRISSYDLE
ILRDDKKFFPPYEASMVVNNSIIKKDPKLKKLLHRLDGKINLKTMQNLNYMVDDKLLEPSVVAKQFLEKNHYFRGDK
```

SeqID 476

```
LKPSFKKLLLLFSIITILSIACTPHAKASGRSWKSWFIEQYFWLKRDKSYYKVQDESSFQKYLNASREQSDKGYYLD
PNSVNGGLVQERLFDMQVYSWNDNGKANQKTIFYLAGGSYLNNPTPYHISMLKTLSTSLDAKIILPIYPKTPRYTYD
YAIPRLVNLYRHFHEKNANLTLMGDSAGGGLALGLAHALSHQSGQEAIPQPKNIILLSPWLDVTMKHPEIPKYEDTD
PILSAWGLARVGEIWANGSNNTNHTYVSPKNAPATKLAPITLFTGTREIFFPDIRDYAAQLQAANHPVNYIAQEGMN
HVYPIYPIEEAKTAQYQMIDIINKTP
```

SeqID 477

```
MTKKHLKTLALALTTVSVVTYSQEVYGLEREESVKQEQTQSASEDDWFEEDNERKTNVSKGNSTVDETVSDLFSDGN
SNNSSSKTESVVSDPKQVPKAKPEVTQEASNSSNDASKVEVPKQDTASKKETIETSTWEAKDFVTRGDTLVGFSKSG
INKLSQTSHLVLPSHAADGTQLTQVASFAFTPDKKTAIAEYTSRLGENGKPSRLDIDQKEIIDEGEIFNAYQLTKLT
IPNGYKSIGQDAFVDNKNIAEVNLPESLETISDYAFAHMSLKQVKLPDNLKVIGELAFFDNQIGGKLYLPRHLIKLA
ERAFKSNRIQTVEFLGSKLKVIGEASFQDNNLSNVMLPDGLEKIESEAFTGNPGDEHYNNQVVLRTRTGQNPHQLAT
ENTYVNPDKSLWRATPDMDYTKWLEEDFTYQKNSVTGFSNKGLQKVRRNKNLEIPKQHNGITITEIGDNAFRNVDFQ
SKTLRKYDLEEVKLPSTIRKIGAFAFQSNNLKSFEASEDLEEIKEGAFMNNRIGTLDLKDKLIKIGDASFHINHIHA
IVLPESVQEIGRSAFRQNGALHLMFIGNKVKTIGEMAFLSNKLESVNLSEQKQLKTIEVQAFSDNALSEVVLPPNLQ
TIREEAFKRNHLKEVKGSSTLSQIAFNAFDQNDGDKRFGKKVVVRTHNNSHMLADGERFIIDPDKLSSTMVDLEKVL
KIIEGLDYSTLRQTTQTQFREMTTAGKALLSKSNLRQGEKQKFLQEAQFFLGRVDLDKATAKAEKALVTKKATKNGH
LLERSINKAVLAYNNSAIKKANVKRLEKELDLLTDLVEGKGPLAQATMVQGVYLLKTPLPLPEYYIGLNVYFDKSGK
LIYALDMSDTIGEGQKDAYGNPILNVDEDNEGYHALAVATLADYEGLYIKDILNSSLDKIKAIRQIPLAKYHRLGFF
QAIRNAAAEADRLIPKTPKGYLNTVPNYRKKQVEKNSKPVDYKTPIFDKALPNEKVDGDRVAKGHNINAETNNYVAV
TPIRSEQQLHKSQSDVNLPQTSSKNNFIYEILGYVSLCLLFLVTAGKKGKRARK
```

SeqID 478

```
MNKKIAGIGLASIAVLSLAACGHRGASKSGGKSDSLKVAMVTDTGGVDDKSFNQSGWEGMQAWGKKNGLKKGAGFDY
FQSASESDYATNLDTAVSSGYKLIFGIGFSLHDAIDKAADNNKDVNYVIVDDVIKGKDNVASVVFADNESAYLAGIA
AAKTTKTKTVGFVGGMESEVITRFEKGFEAGVKSVDKSIKIKVDYAGSFGDAAKGKTIAAAQYASGADIVYQVAGGT
GAGVFSEAKSRNESLKEADKVWVLGVDRDQAAEGKYTSKDGKASNFVLASSIKEVGKSVELIATKTSKGKFPGGNVT
TYGLKDGGVDIATTNLSDDAVKAIKEAKAKIISGDIKVPSK
```

SeqID 479

```
MRIQTTNLDKGGGGKSSHTYNEGDWLSRVMGKRVLLIDGARECNLTHSFDVTSDKTIYDIFITGEYEICRINDKLSL
IRGDERLTDEQLDLASRNNKYLQLFMWFSEHYEELAEQFDIILIDTHNDESLVTANCIAVSDIVLGVTDASTNGFRA
WLALKKFVDDIKNEAIEVITKKSYVKAEAYLIGNKIEYYGNNVTETCKQFLDVVQEDEAYLGSIQKKELMAKSLVIN
QSVFEQREHMTEKQKQDHQKFYDNIEYVYQNILTVLEGETK
```

SeqID 480

```
VKKVVFLKILVPLVSLVFLISLGITILAGVLGAVGGSQSNCTIEVVTSTSTDSSISSGDGSIDSFVKEHKEAYILSW
KAGGFLPSASITQTMIENGFNFTNPNGTSLWQAHNMGGVKTSSKSNFPVTLATYGQDSVDLSGTKPGARVGDGTGGA
YTWFSSYDAGIVGKAEFMAHQTLYTKAINNIDGIATLSAIADGGWATDGTYKTKLIDMYHKLGKTYEWLDKEAISAH
GDKPYKATTASAGDAGLSDIFTSNSKDCSDSSSGGATDGTGTVPADAIAWGYRPDNLPESLKQYIIDPKSLGMAYAN
SSGWFNPGSDYLAGQCVNLTISLGNHLWGHSGSVSGNGKDQAGAWANIFGNSVKTTPKRGAIFSTQNGGGGYGHTGI
VCHVFENGSILIVEQNTPLSGADYFKKSYTWNYRIWTPAQQKAEVISFAYPYGKEPKLGN
```

SeqID 481
MGKTFWKDIYRSITTSKGRFSSILLLMMLGSFAFIGLKVSAPNMQRTAQNYLAHHHVMDITVFNSWGLDKHDQTVLE
SLKGSQVEFSYFVDTTPQQNSKSYRLYSNTKTISTFDLVKGRLPLNKSEIALSFQERKKYAIGDKINFKQDKNKLFS
NTGPLTIVGFVNSTEIWSKTNLGSSQTGDGDLDSYGVLDKTAFHSPVYTMARVTFKDLRLINPFSISYKEKVAKYQE
KVSRKLNIHNKIRYTKTKKESLRKIDEEEKSLLKAQKQINRLDNDSLAMPLSQRQAIQMKIKQDRLSLLKRTKELLK
LRHNTQIMESPQIIVYNRTTFPGGQGYNTFDSSTNSTSKISNLFPIILYLVAALVTLTTMTRFVEEERTNAGILKAL
GYSDRQVIFKFIIYGFIAGTLGTTLGIIGGHYLLPRIISDIISKDLTIPNTQYHLFLNYSLLAFVFSLLSIVLPVFV
ITRRELKEKAAFLLLPKPPAKGSKIALEYINWIWKKLSFTQKVTARNIFRYKQRMIMTIFGVAGSVALLFSGLGIQS
SLKQTVNEHFGRIMPYDILLTYNTNASPPKILELLSKDSKIDKYQPIHLENLDESIPGQINKQSISLFITDKKQLLP
FIYLQEATTNKSLHLNNKGIIISKKLAQFYHVNTGDFIHLSHSQTLPSRKLKITGVVNANVGHYIFMTKQYYRTIFK
KETKDNAFLVKLTKHKIANNLAEKLLEINGVESLTQNALQLASVEAVVRSLDGSMTILVVVSLLLAIVILYNLTNIN
LAERKRELSTIKVLGFYNEEVTLYIYRETIILSTIGVILGTISGTYLHRQMMLLIGSDQILFGEKVSPTTFIIPISV
VVIILISLCFIVNHQLKKLNMLDALKSVD

SeqID 482
MNKRKLLTVLSIASVAVLSSVTFADEITPSDPTAPSTPLITPTPVDPGTPTDTTKPSTPVDPGTPTDTTKPSTPVDP
GTPTDTTKPSTPVDSGTSTNPSDSEATNTNLGTKPTDVVKPETPKIPEVPSTVTPDVIDKVDKETGNITIKPIQISP
DKTIIGTQNGNVIIRDSSGSHLVSASEVGGRLNEDGTVTIKDSSGKTKTLPRTGEAKGFLSIIGATILSFVAYLFKQ
RVTLN

SeqID 483
MRPIVRKKMYKKGKFLVIAGIVTILGGSAILGQDVKAEQAEAATSTISEKTDSSQTISDTSKLTLPVNSSEAMKNSA
EPLIKTGFATSVSSNPREIAATPVKTFDASSKVVVKASTAEHSANQTNSNVNQVANDSEVITQQNSTKQLPKVTYSA
HVQDIGWQKSVDNATVSGTVGQEKQVEAIKLSIKAPEGITGKLSYKTYVKGQGWQPSVESGQVSGTVGQSRPIEALS
INLTDNLQKLYDVYYRVHVQDIGWMAWAKNGAYAGTLGMSKRLEAYEVKFTLKGQSVLTPTILKEERPVLNYQVKVG
QNGWQSNKLEGQMAGTLGESKALDGVKFTLSTLKYGDILYRTHVQDKGWGEILGSQTSKDYQGKRLEAIQLDLSGNL
KQNYDIYYRAHVQDKGWMAWQKNNSVAGTVGESKRLEALEVKLVAKASDFSSQTNHSFLEMKHPGLSYQTYLQKDGW
KPTVLEGQLGGSIGLSKSIKAIKLNLGSTALGNIEYRTFLNGSGWQTVVNSGRESNVPNESQQVEAIQMRLTGLLSK
TYSVMYRVHMQDYGWQDWTYNNDIAGSTNQGKRIEAIEIKLVETAKIPITVQTTYKGTGNYNVRVTNVISPGNLKIA
IWSDKNNQDDLKWYTVTPKNHEARLTFNVTNHRDNGKYFIHVYQENASNQKQLLVKTSLQVAHSNYNTPYFSQRDGR
WASRRYGMATLGETGCVPTSLAMILSSLKGETVLPTQIADYLYHKTVEFNRGVQGTTSRGILKAAKEWGVTATALGT
QANLVQALKDGYHVLAAVQNNVFVLHGSHEIVLKGYNNGLTHVSDPYTPSLSGWYPISQLWKEQSYYSEDRIDIGEP
FVKVTDA

SeqID 484
MARIPYQKTLIFCVAVIIAIFILGLSKDLAQSKGQKVANNNTVKTARVVANGDILLHDVLYASARQPDGTYNFTPYF
KEVKSWIESADLAIGDYEGTISSEYPLAGYPLFNAPNEIATTMKETGYDVVDLAHNHILDSQLAGAINTVKTFNRMG
LDTIGVYLKDRNKEDILIKRVNGIKIAILGYSYGYNGMEANVSKSDYEKHMSDLDTKKIKQDIKKAEKEADITIVMP
QMGIEYQKKPTTEQVMLYHSMIKWGADIIFGGHPHVVEPSEVIKKDGQKKFIIYSMGNFISNQRLETVDDIWTERGL
LMDVTIEKKGQKTVIKKVKAHPTLVEAKANGRYVEEGFPAYDYRTLVLEDYIEGGKKRSTIDQTMKDKVDKAYKEMN
ELVNLKW

SeqID 485
MKHFFDYKTMSVARKLSISFIAVILLGSILLSLPIFQYANAPKTHYIDHLFTTVSMVCVTGLSVFPISKVYNGWGQI
VAILLMQTGGLGLVTLMSLSYYTLRRKMSLNDQTLLQSAITYNSSTDLKKYLYMIFKVTLTLEVLAASILAIDFIPR
FGLGHGIFNSIFLAVSAFCNAGFDNLGATSLAQFKLNPLVNIIVCFLIISGGLGFAVWKDLIEATIQTSHKGPKLIK
TFPKRLSNHSKLVLKTTTIILLTGTLLSWLLEFGNFRTIANLSLPKQLMVSFFQTVTMRTAGFSTIDYTQTDFATNL
VYIIQMLIGGAPGGTAGGFKVTVIAILLLLFKAELSGQSQVTFHYRTIPSSIIKQTLSILTFFFIILISGYLLLLEL
NPHIDPFSLFFEASSALATVGVTMNTTNQLSLGGRIVIMFLMFIGRVGPITVLLSILQKKEKEIHYAETEIILG

SeqID 486
MLDELKAIIKSPKLWITMAGVALIPTLYNVIFLSSMWDPYGNTKNLPVAVVNQDKSAKLNGKTISIGKDMEDNLSKN
DSLDFHFTTAKRAEKELEKGHYYMVITFPKDLSRKATTLMTEKPERLNITYKTTKGRSFVASKMSETAANKLKDEVA
ESITGTYTESVFKNMGSMKTGINKAADGSQELLNGSNKLQDGSQTLTSNLDVLASSSQTFSGGANKLNSGINLYTDG
VGTLSNGLETLSDGVTAYTTGVHKLSEGSQKLDDKSQALVEGSEKLTDGLQQLSQATQLKPEQERTLQNLSDGLKNL
NQIITNLQSTATTDSDTNSKLFNFLSTIESSTKALMNTAAADKQKQMTAVQSTSAFKSLTPEQQSQITSAVTGTPTS
AETIAANISSNIENMKTVLSEASSSAPSNNGSQNLQTLSGTANNLVLKAISDLDKIQKLPTATKQLYQGSQTLTKGI
TDYTNAVGQLRKGAVTLDSKSNQLISGTQKASQGAQTLDSKSDQLRDGAGQLASGSDRIADGSNKLAGGGHQLIDGL
TELSGGVSQLSSSLGKAGDQLSMVSVNKDNANAVSSPVTIKHEDYDSVDTNGVGMAPYMISVALMVVALSANVIFAK
ALSGKEPANRFSWAKNKLLINGFIATLAATILFFAVQFIGLKPDYPGKTYFIILLTAWTLMALVTALVGWDNRYGSF
LSLLILLFQLGSSAGTYPIELSPKFFQTIQPFLPMTYSVSGLRETISLTGDVNHQWRMLVIFLVSSMILALLIYRKQ
ED